PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 7/06, 7/08

A2

(11) International Publication Number: WO 98/40401

(43) International Publication Date: 17 September 1998 (17.09.98)

(21) International Application Number: PCT/CA98/00190

(22) International Filing Date: 10 March 1998 (10.03.98)

(30) Priority Data:

60/040,649	10 March 1997 (10.03.97)	US
08/915,314	20 August 1997 (20.08.97)	US
60/060,099	26 September 1997 (26.09.97)	US
09/030,619	25 February 1998 (25.02.98)	US

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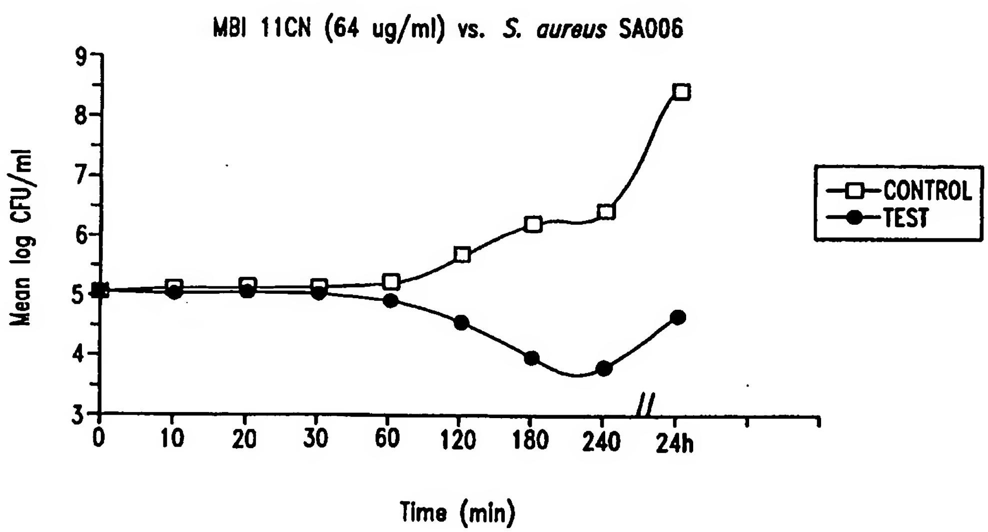
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOSITIONS AND METHODS FOR TREATING INFECTIONS USING CATIONIC PEPTIDES ALONE OR IN COMBINATION WITH ANTIBIOTICS



(57) Abstract

Compositions and methods for treating infections, especially bacterial infections, are provided. Cationic peptides in combination with an antibiotic agent are administered to a patient to enhance the activity of the antibiotic agent, overcome tolerance, overcome acquired resistance, or overcome inherent resistance.

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COMPOSITIONS AND METHODS FOR TREATING INFECTIONS USING CATIONIC PEPTIDES ALONE OR IN COMBINATION WITH ANTIBIOTICS

TECHNICAL FIELD

The present invention relates generally to methods of treating microorganism-caused infections using cationic peptides or a combination of cationic peptides and antibiotic agents, and more particularly to using these peptides and antibiotic agents to overcome acquired resistance, tolerance, and inherent resistance of an infective organism to the antibiotic agent.

BACKGROUND OF THE INVENTION

For most healthy individuals, infections are irritating, but not generally life-threatening. Many infections are successfully combated by the immune system of the individual. Treatment is an adjunct and is generally readily available in developed countries. However, infectious diseases are a serious concern in developing countries and in immunocompromised individuals.

In developing countries, the lack of adequate sanitation and consequent poor hygiene provide an environment that fosters bacterial, parasitic, fungal and viral infections. Poor hygiene and nutritional deficiencies may diminish the effectiveness of natural barriers, such as skin and mucous membranes, to invasion by infectious agents or the ability of the immune system to clear the agents. As well, a constant onslaught of pathogens may stress the immune system defenses of antibody production and phagocytic cells (*e.g.*, polymorphic neutrophils) to subnormal levels. A breakdown of host defenses can also occur due to conditions such as circulatory disturbances, mechanical obstruction, fatigue, smoking, excessive drinking, genetic defects, AIDS, bone marrow transplant, cancer, and diabetes. An increasingly prevalent problem in the world is opportunistic infections in individuals who are HIV positive.

Although vaccines may be available to protect against some of these organisms, vaccinations are not always feasible, due to factors such as inadequate delivery

mechanisms and economic poverty, or effective, due to factors such as delivery too late in the infection, inability of the patient to mount an immune response to the vaccine, or evolution of the pathogen. For other pathogenic agents, no vaccines are available. When protection against infection is not possible, treatment of infection is generally pursued. The major weapon in the arsenal of treatments is antibiotics. While antibiotics have proved effective against many bacteria and thus saved countless lives, they are not a panacea. The overuse of antibiotics in certain situations has promoted the spread of resistant bacterial strains. And of great importance, antibacterials are useless against viral infections.

A variety of organisms make cationic (positively charged) peptides, molecules used as part of a non-specific defense mechanism against microorganisms. When isolated, these peptides are toxic to a wide variety of microorganisms, including bacteria, fungi, and certain enveloped viruses. One cationic peptide found in neutrophils is indolicidin. While indolicidin acts against many pathogens, notable exceptions and varying degrees of toxicity exist.

In addition neither antibiotic therapy alone of cationic peptide therapy alone can effectively combat all infections. By expanding the categories of microorganisms that respond to therapy, or by overcoming the resistance of a microorganism to antibiotic agents, health and welfare will be improved. Additionally quality of life will be improved, due to, for example, decreased duration of therapy, reduced hospital stay including high-care facilities, with the concomitant reduced risk of serious nosocomial (hospital-acquired) infections.

The present invention discloses cationic peptides, including analogues of indolicidin and cecropin/melittin fusion peptides, in combination with antibiotics such that the combination is either synergistic, able to overcome microorganismal tolerance, able to overcome resistance to antibiotic treatment, or further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention generally provides the co-administration of cationic peptides with an antibiotic agent and also provides specific indolicidin analogues.

In other embodiments, the cationic peptide analogue has one or more amino acids altered to a corresponding D-amino acid, and in certain preferred embodiments, the N-terminal and/or the C-terminal amino acid is a D-amino acid. Other preferred modifications include analogues that are acetylated at the N-terminal amino acid, amidated at the C-terminal amino acid, esterified at the C-terminal amino acid, and modified by incorporation of homoserine/homoserine lactone at the C-terminal amino acid. In other aspects, a composition is provided, comprising an indolicidin analogue and an antibiotic.

In addition, a device, which may be a medical device, is provided that is coated with a cationic peptide and an antibiotic agent.

This invention also generally provides methods for treating infections caused by a microorganism using a combination of cationic peptides and antibiotic agents. In one aspect, the method comprises administering to a patient a therapeutically effective dose of a combination of an antibiotic agent and a cationic peptide, wherein administration of an antibiotic agent alone is ineffective. Preferred antibiotics and peptides are provided.

In another aspect, a method of enhancing the activity of an antibiotic agent against an infection in a patient caused by a microorganism is provided, comprising administering to the patient a therapeutically effective dose of the antibiotic agent and a cationic peptide. In yet another aspect, a method is provided for enhancing the antibiotic activity of lysozyme or nisin, comprising administering lysozyme or nisin with an antibiotic agent.

In other aspects, methods of treating an infection in a patient caused by a bacteria that is tolerant to an antibiotic agent, caused by a microorganism that is inherently resistant to an antibiotic agent; or caused by a microorganism that has acquired resistance to an antibiotic agent; comprises administering to the patient a therapeutically effective dose of the antibiotic agent and a cationic peptide, thereby overcoming tolerance, inherent or acquired resistance to the antibiotic agent.

In yet other related aspects, methods are provided for killing a microorganism that is tolerant, inherently resistant, or has acquired resistance to an antibiotic agent, comprising contacting the microorganism with the antibiotic agent and a cationic peptide,

thereby overcoming tolerance, inherent resistance or acquired resistance to the antibiotic agent.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures or compositions, and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-E present time kill assay results for MBI 11CN, MBI 11F3CN, MBI 11B7CN, MBI 11F4CN, and MBI 26 plus vancomycin. The number of colony forming units x 10^{-4} is plotted versus time.

Figure 2 is a graph showing the stability of MBI11B7CN in heat-inactivated rabbit serum.

Figure 3 presents HPLC tracings showing the effects of amastatin and bestatin on peptide degradation.

Figure 4 is a chromatogram showing extraction of peptides in rabbit plasma.

Figure 5 is a graph presenting change in *in vivo* MBI 11CN levels in blood at various times after intraperitoneal injection.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that are used herein.

The amino acid designations herein are set forth as either the standard one-or three-letter code. A capital letter indicates an L-form amino acid; a small letter indicates a D-form amino acid.

As used herein, an "antibiotic agent" refers to a molecule that tends to prevent, inhibit, or destroy life. The term "antimicrobial agent" refers to an antibiotic agent specifically directed to a microorganism.

As used herein, "cationic peptide" refers to a peptide that has a net positive charge within the pH range of 4-10. A cationic peptide is at least 5 amino acids in length and has at least one basic amino acid (*e.g.*, arginine, lysine, histidine). Preferably, the peptide has measurable anti-microbial activity when administered alone.

As used herein, a "peptide analogue", "analogue", or "variant" of a cationic peptide, such as indolicidin, is at least 5 amino acids in length, has at least one basic amino acid (*e.g.*, arginine and lysine) and has anti-microbial activity. Unless otherwise indicated, a named amino acid refers to the L-form. Basic amino acids include arginine, lysine, histidine and derivatives. Hydrophobic residues include tryptophan, phenylalanine, isoleucine, leucine, valine, and derivatives.

Also included within the scope of the present invention are amino acid derivatives that have been altered by chemical means, such as methylation (*e.g.*, α methylvaline), amidation, especially of the C-terminal amino acid by an alkylamine (*e.g.*, ethylamine, ethanolamine, and ethylene diamine) and alteration of an amino acid side chain, such as acylation of the ε-amino group of lysine. Other amino acids that may be incorporated in the analogue include any of the D-amino acids corresponding to the 20 L-amino acids commonly found in proteins, imino amino acids, rare amino acids, such as hydroxylysine, or non-protein amino acids, such as homoserine and ornithine. A peptide analogue may have none or one or more of these derivatives, and D-amino acids. In addition, a peptide may also be synthesized as a retro-, inverto- or retro-inverto-peptide.

As used herein "inherent resistance" of a microorganism to an antibiotic agent refers to a natural resistance to the action of the agent even in the absence of prior exposure to the agent. (R.C. Moellering Jr., *Principles of Anti-infective Therapy; In: Principles and Practice of Infectious Diseases*, 4th Edition, Eds.; G.L. Mandell, J.E. Bennett, R. Dolin. Churchill Livingstone, New York USA, 1995, page 200).

As used herein, "acquired resistance" of a microorganism to an antibiotic agent refers to a resistance that is not inhibited by the normal achievable serum concentrations of a recommended antibiotic agent based on the recommended dosage. (NCCLS guidelines).

As used herein, "tolerance" of a microorganism to an antibiotic agent refers to when there is microstatic, rather than microcidal effect of the agent. Tolerance is measured by an MBC:MIC ratio greater than or equal to 32. (*Textbook of Diagnostic Microbiology*, Eds., C.R. Mahon and G. Manuselis, W.B. Saunders Co., Toronto Canada, 1995, page 92).

As noted above, this invention provides methods of treating infections caused by a microorganism, methods of killing a microorganism, and methods of enhancing the activity of an antibiotic agent. In particular, these methods are especially applicable when a microorganism is resistant to an antibiotic agent, by a mechanism, such as tolerance, inherent resistance, or acquired resistance. In this invention, infections are treated by administering a therapeutically effective dose of a cationic peptide alone or in combination with an antibiotic agent to a patient with an infection. Similarly, the combination can be contacted with a microorganism to effect killing.

I. CATIONIC PEPTIDES

As noted above, a cationic peptide is a peptide that has a net positive charge within the pH range 4-10. A peptide is at least 5 amino acids long and preferably not more than 25, 27, 30, 35, or 40 amino acids. Peptides from 12 to 30 residues are preferred. Examples of native cationic peptides include, but are not limited to, representative peptides presented in the following table.

Table 1. Cationic Peptides

Group Name	Peptide	Origin	Sequence	Accession Number	Reference*
Abaecins	Abaecin	Honey bee *(Apis mellifera)*	YVPLPNVPQPGRRPFPTFPGQGPFNPKIK WPQGY	P15450	Casteels P. et al., (1990)
Andropins	Andropin	Fruit fly *(Drosophilia melanogaster)*	VFIDILDKVENAIHNAAQVGIGFAKPFEKL INPK	P21663	Samakovlis, C. et al., (1991)
Apidaecins	Apidaecin IA	Lymph fluid of honey bee *(Apis mellifera)*	GNNRPVYIPQPRPPHPRI	P11525	Casteels, P. et al., (1989)
	Apidaecin IB	"	GNNRPVYIPQPRPPHPRL	P11526	Casteels, P. et al., (1989)
	Apidaecin II	"	GNNRPIYIPQPRPPHPRL	P11527	Casteels, P. et al., (1989)
AS	AS-48	*Streptococcus faecalis* subsp. *Liquefacines* S-48	7.4 kDa		Galvez, A., et al., (1989)
Bactenecins	Bactenecin	Cytoplasmic granules of bovine neutrophils	RLCRIVVIRVCR	A33799	Romeo, D. et al., (1988)
Bac	Bac5	Cytoplasmic granules of bovine neutrophils	RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPP FRPPLRFP	B36589	Frank, R.W. et al., (1990)
	Bac7	"	RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPL PFPRPGPRPIPRPLPFPRPGPRPIPRP	A36589	Frank, R.W. et al., (1990)
Bactericidins	Bactericidin B2	Tobacco hornworm larvae hemolymph *(Manduca sexta)*	WNPFKELERAGQRVRDAVISAAPAVATV GQAAAIARG*	P14662	Dickinson, L. et al., (1988)
	Bactericidin B-3	"	WNPFKELERAGQRVRDAIISAGPAVATV GQAAAIARG	P14663	Dickinson, L. et al., (1988)
	Bactericidin B-4	"	WNPFKELERAGQRVRDAIISAAPAVATV GQAAAIARG*	P14664	Dickinson, L. et al., (1988)
	Bactericidin B-5P	"	WNPFKELERAGQRVRDAVISAAAVATVG QAAAIARGG*	P14665	Dickinson, L., et al., (1988)
Bacteriocins	Bacteriocin C3603	*Streptococcus mutants*	4.8 kDa		Takada, K., et al., (1984)
	Bacteriocin IY52	*Staphylococcus aureus*	5 kDa		Nakamura, T., et al., (1983)

Group Name	Peptide	Origin	Sequence	Accession Number	Reference*
Bombinins	Bombinin	Yellow-bellied toad *(Bombina variegata)*	GIGALSAKGALKGLAKGLAZHFAN*	P01505	Csordas, A., and Michl, H. (1970)
	BLP-1	Asian Toad *(Bombina orientalis)*	GIGASILSAGKSALKGLAKGLAEHFAN*	M76483	Gibson, B.W. et al., (1991)
	BLP-2	"	GIGSAILSAGKSALKGLAKGLAEHFAN*	B41575	Gibson, B.W. et al., (1991)
Bombolitins	Bombolitin BI	Bumblebee venom *(Megabombus pennsylvanicus)*	IKITTMLAKLGKVLAHV*	P10521	Argiolas, A. and Pisano, J.J. (1985)
	Bombolitin BII	"	SKITDILAKLGKVLAHV*	P07493	Argiolas, A. and Pisano, J.J. (1985)
BPTI	Bovine Pancreatic Trypsin Inhibitor (BPTI)	Bovine Pancreas	RPDFCLEPPYTGPCKARIIRYFYNAKAGL CQTFVYGGCRAKRNNFKSAEDCMRTCG GA	P00974	Creighton, T. and Charles, I.G. (1987)
Brevinins	Brevinin-1E	European frog *(Rana esculenta)*	FLPLLAGLAANFLPKIFCKITRKC	S33729	Simmaco, M. et al., (1993)
	Brevinin-2E		GIMDTLKNLAKTAGKGALQSLLNKASCK LSGQC	S33730	Simmaco, M. et al., (1993)
Cecropins	Cecropin A	Silk moth *(Hyalophora cecropia)*	KWKLFKKIEKVGQNIRDGIIKAGPAVAVV GQATQIAK*	M63845	Gudmundsson, G.H. et al., (1991)
	Cecropin B	Silk moth (Hyalophora cecropia)	KWKVFKKIEKMGRNIRNGIVKAGPAIAV LGEAKAL*	Z07404	Xanthopoulos, G. et al. (1988)
	Cecropin C	Fruit fly *(Drosophila melanogaster)*	GWLKKLGKRIERIGQHTRDATIQGLGIAQ QAANVAATARG*	Z11167	Tryselius, Y. et al. (1992)
	Cecropin D	Silk moth pupae *(Hyalophora cecropia)*	WNPFKELEKVGQRVRDAVISAGPAVATV AQATALAK*	P01510	Hultmark, D. et al., (1982)
	Cecropin P_1	Pig small intestine *(sus scrofa)*	SWLSKTAKKLENSAKKRISEGIAIAIQGGP R	P14661	Lee, J.-Y. et al., (1989)

Group Name	Peptide	Origin	Sequence	Accession Number	Reference*
Charybdtoxins	Charybdtoxin	Scorpion venom *(Leiurus quin-questriatus hebraeus)*	ZFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS	P13487	Schweitz, H. et al., (1989)
Coleoptericins	Coleoptericin	Beetle *(Zophobas atratus)*	8.1 kDa	A41711	Bulet, P. et al., (1991)
Crabolins	Crabolin	European hornet venom *(Vespa crabo)*	FLPLILRKIVTAL*	A01781	Argiolas, A. and Pisano, J.J. (1984)
Defensins-alpha	Cryptdin 1	Mouse intestine *(Mus musculus)*	LRDLVCYCRSRGCKGRERMNGTCRKGHLLYTLCCR	A43279	Selsted, M.E. et al., (1992)
	Cryptdin 2	"	LRDLVCYCRTRGCKRRERMNGTCRKGHLMYTLCCR	C43279	Selsted, M.E. et al., (1992)
	MCP1	Rabbit alveolar macrophages *(Oryctolagus cuniculus)*	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR	M28883	Selsted, M. et al., (1983)
	MCP2	"	VVCACRRALCLPLERRAGFCRIRGRIHPLCCRR	M28073	Ganz, T. et al., (1989)
	GNCP-1	Guinea pig *(Cavia cutteri)*	RRCICTTRTCRFPYRRLGTCIFQNRVYTFCC	S21169	Yamashita, T. and Saito, K., (1989)
	GNCP-2	"	RRCICTTRTCRFPYRRLGTCLFQNRVYTFCC	X63676	Yamashita, T. and Saito, K., (1989)
	HNP-1	Azurophil granules of human neutrophils	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	P11479	Lehrer, R. et al., (1991)
	HNP-2	"	CYCRIPACIAGERRYGTCIYQGRLWAFCC	P11479	Lehrer, R. et al., (1991)
	NP-1	Rabbit neutrophils *(Oryctolagus cuniculus)*	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR	P01376	Ganz, T. et al., (1989)
	NP-2	"	VVCACRRALCLPLERRAGFCRIRGRIHPLCCRR	P01377	Ganz, T. et al., (1989)
	RatNP-1	Rat neutrophils *(Rattus norvegicus)*	VTCYCRRTRCGFRERLSGACGYRGRIYRLCCR	A60113	Eisenhauer, P.B. et al., (1989)

Group Name	Peptide	Origin	Sequence	Accession Number	Reference*
	RatNP-2	"	VTCYCRSTRCGFRERLSGACGYRGRIYRL CCR		Eisenhauer, P.B. et al., (1989)
Defensins-beta	BNBD-1	Bovine neutrophils	DFASCHTNGGICLPNRCPGHMIQIGICFRP RVKCCRSW	127951	Selsted, M.E. et at., (1993)
	BNBD-2	"	VRNHVTCRINRGFCVPIRCPGRTRQIGTCF GPRIKCCRSW	127952	Selsted, M.E., et al., (1993)
	TAP	Bovine tracheal mucosa *(Bos taurus)*	NPVSCVRNKGICVPIRCPGSMKQIGTCVG RAVKCCRKK	P25068	Diamond, G. et al., (1991)
Defensins-insect	Sapecin	Flesh fly *(Sacrophaga peregrina)*	ATCDLLSGTGINHSACAAHCLLRGNRGG YCNGKAVCVCRN	J04053	Hanzawa, H. et al., (1990)
	Insect defensin	Dragonfly larvae *(Aeschna cyanea)*	GFGCPLDQMQCHRHCQTITGRSGGYCSG PLKLTCTCYR	P80154	Bulet, P. et al., (1992)
Defensins-scorpion	Scorpion defensin	Scorpion *(Leiurus quinquestriatus)*	GFGCPLNQGACHRHCRSIRRRGGYCAGF FKQTCTCYRN		Cociancich, S. et al., (1993)
Dermaseptins	Dermaseptin	South American arboreal frog *(Phyllomedusa sauvagii)*	ALWKTMLKKLGTMALHAGKAALGAAD TISQTQ	P24302	Mor, A., et al., (1991)
Diptericins	Diptericin	Nesting-suckling blowfly *(Phormia terranovae)*	9 kDa	X15851	Reichhardt, J.M. et al., (1989)
Drosocins	Drosocin	Fruit fly *(Drosophila melanogaster)*	GKPRPYSPRPTSHPRPIRV	S35984	Bulet, P. et al., (1993)
Esculentins	Esculentin	European frog *(Rana esculenta)*	GIFSKLGRKKIKNLLISGLKNVGKEVGMD VVRTGIDIAGCKIKGEC	S33731	Simmaco, M. et al., (1993)
Indolicidins	Indolicidin	Bovine neutrophils	ILPWKWPWWPWRR*	A42387	Selsted, M. et al., (1992)
Lactoferricins	Lactoferricin B	N terminal region of bovine lactoferrin	FKCRRWQWRMKKLGAPSITCVRRAF	M63502	Bellamy, W. et al., (1992b)
Lantibiotics	Nisin	*Lactococcus lactis* subsp. *Lactis* (bacterium)	ITSISLCTPGCKTGALMGCNMKTATCHCS IHVSK	P13068	Hurst, A. (1981)
	Pep 5	*Staphylococcus epidermidis*	TAGPAIRASVKQCQKTLKATRLFTVSCKG KNGCK	P19578	Keletta, C. et al., (1989)

Group Name	Peptide	Origin	Sequence	Accession Number	Reference*
	Subtilin	*Bacillus subtilis* (bacterium)	MSKFDDFDLDVVKVSKQDSKITPQWKSE SLCTPGCVTGALQTCFLQTLTCNCKISK	P10946	Banerjee, S. and Hansen, J.N. (1988)
Leukocins	Leukocin A-val 187	*Leuconostoc gelidum* UAL 187 (bacterium)	KYYGNGVHCTKSGCSVNWGEAFSAGVH RLANGGNGFW	S65611	Hastings, J.W. et al., (1991)
Magainins	Magainin I	Amphibian skin *(Xenopus laevis)*	GIGKFLHSAGKFGKAFVGEIMKS*	A29771	Zasloff, M. (1987)
	Magainin II	"	GIGKFLHSAKKFGKAFVGEIMNS*	A29771	Zasloff, M. (1987)
	PGLa	Amphibian skin *(Xenopus laevis)*	GMASKAGAIAGKIAKVALKAL*	X13388	Kuchler, K. et al., (1989)
	PGQ	Amphibian stomach *(Xenopus laevis)*	GVLSNVIGYLKKLGTGALNAVLKQ		Moore, K.S. et al., (1989)
	XPF	Amphibian skin *(Xenopus laevis)*	GWASKIGQTLGKIAKVGLKELIQPK	P07198	Sures, I. And Crippa, M. (1984)
Mastoparans	Mastoparan	Wasp venom *(Vespula lewisii)*	INLKALAALAKKIL*	P01514	Bernheimer, A. and Rudy, B. (1986)
Melittins	Melittin	Bee venom *(Apis mellifera)*	GIGAVLKVLTTGLPALISWIKRKRQQ	P01504	Tosteson, M.T. and Tosteson, D.C.(1984)
Phormicins	Phormicin A	Nestling-suckling blowfly *(Phormia terranovae)*	ATCDLLSGTGINHSACAAHCLLRGNRGG YCNGKGVCVCRN	P10891	Lambert, J. et al., (1989)
	Phormicin B	"	ATCDLLSGTGINHSACAAHCLLRGNRGG YCNRKGVCVRN	P10891	Lambert, J. et al., (1989)
Polyphemusins	Polyphemusin I	Atlantic horseshoe crab *(Limulus polyphemus)*	RRWCFRVCYRGFCYRKCR*	P14215	Miyata, T. et al., (1989)
	Polyphemusin II	"	RRWCFRVCYKGFCYRKCR*	P14216	Miyata, T. et al., (1989)
Protegrins	Protegrin I	Porcine leukocytes *(sus scrofa)*	RGGRLCYCRRRFCVCVGR	S34585	Kokryakov, V.N. et al., (1993)
	Protegrin II	"	RGGRLCYCRRRFCICV	S34586	Kikryakov, V.N. et al., (1993)

Group Name	Peptide	Origin	Sequence	Accession Number	Reference*
	Protegrin III	"	RGGGLCYCRRRFCVCVGR	S34587	Kokryakov, V.N. et al., (1993)
Royalisins	Royalisin	Royal Jelly *(Apis mellifera)*	VTCDLLSFKGQVNDSACAANCLSLGKAG GHCEKGVCICRKTSFKDLWDKYF	P17722	Fujiwara, S. et al., (1990)
Sarcotoxins	Sarcotoxin IA	Flesh fly *(Sacrophaga peregrina)*	GWLKKIGKKIERVGQHTRDATIQGLGIAQ QAANVAATAR*	P08375	Okada, M. and Natori S., (1985b)
	Sarcotoxin IB	"	GWLKKIGKKIERVGQHTRDATIQVIGVA QQAANVAATAR*	P08376	Okada, M. and Natori S., (1985b)
Seminal plasmins	Seminalplasmin	Bovine seminal plasma *(Bos taurus)*	SDEKASPDKHHRFSLSRYAKLANRLANP KLLETFLSKWIGDRGNRSV	S08184	Reddy, E.S.P. and Bhargava, P.M. (1979)
Tachyplesins	Tachyplesin I	Horseshoe crab *(Tachypleus tridentatus)*	KWCFRVCYRGICYRRCR*	P23684	Nakamura, T. et al., (1988)
	Tachyplesin II	"	RWCFRVCYRGICYRKCR*	P14214	Muta, T. et al., (1990)
Thionins	Thionin BTH6	Barley leaf *(Hordeum vulgare)*	KSCCKDTLARNCYNTCRFAGGSRPVCAG ACRCKIISGPKCPSDYPK	S00825	Bohlmann, H. et al., (1988)
Toxins	Toxin 1	Waglers pit viper venom *(Trimeresurus wagleri)*	GGKPDLRPCIIPPCHYIPRPKPR	P24335	Schmidt, J.J. et al., (1992)
	Toxin 2	Sahara scorpion *(Androctonus australis Hector)*	VKDGYIVDDVNCTYFCGRNAYCNEECTK LKGESGYCQWASPYGNACYCKLPDHVR TKGPGRCH	P01484	Bontems, F., et al., (1991)

Argiolas and Pisano, (1984). *JBC* 259, 10106; Argiolas and Pisano, (1985). *JBC* 260, 1437; Banerjee and Hansen, (1988). *JBC* 263, 9508; Bellamy et al., (1992). *J. Appl. Bacter.* 73, 472; Bernheimer and Rudy, (1986). *BBA* 864, 123; Bohlmann et al., (1988). *EMBO J.* 7, 1559; Bontems et al., (1991). *Science* 254, 1521; Bulet et al., (1991). *JBC* 266, 24520; Bulet et al. (1992). *Eur. J. Biochem.* 209, 977; Bulet et al., (1993). *JBC* 268, 14893; Casteels et al., (1989). *EMBO J.* 8, 2387; Casteels et al., (1990). *Eur. J. Biochem.* 187, 381; Cociancich et al., (1993). *BBRC* 194, 17; Creighton and Charles, (1987). *J. Mol. Biol.* 194, 11; Csordas and Michl, (1970). *Monatsh Chemistry* 101, 182; Diamond et al., (1991). *PNAS* 88, 3952; Dickinson et al., (1988). *JBC* 263, 19424; Eisenhauer et al., (1989). *Infect. and Imm.* 57, 2021; Frank et al., (1990). *JBC* 265, 18871; Fujiwara et al., (1990). *JBC* 265, 11333; Gálvez et al., (1989). *Antimicrobial Agents and Chemotherapy* 33, 437; Ganz et al., (1989). *J. Immunol.* 143, 1358; Gibson et al., (1991). *JBC* 266, 23103; Gudmundsson et al., (1991). *JBC* 266, 11510; Hanzawa et al., (1990). *FEBS Letters* 269, 413; Hastings et al., (1991). *J. of Bacteriology* 173, 7491; Hultmark et al., (1982). *Eur. J. Biochem.* 127, 207; Hurst, A. (1981). *Adv. Appl. Micro.* 27, 85; Kaletta et al., (1989). *Archives of Microbiology* 152, 16; Kokryakov et al., (1993). *FEBS Letters* 327, 231; Kuchler et al., (1989) *Eur. J. Biochem.* 179, 281; Lambert et al., (1989). *PNAS* 86, 262; Lee et al., (1989). *PNAS* 86, 9159; Lehrer et al., (1991). *Cell* 64, 229; Miyata et al., (1989). *J. of Biochem.* 106, 663; Moore et al., (1991). *JBC* 266, 19851; Mor et al., (1991). *Biochemistry* 30, 8824; Muta et al., (1990). *J. Biochem.* 108, 261; Nakamura et al., (1988). *JBC* 263, 16709; Nakamura et al., (1983). *Infection and Immunity* 39, 609; Okada and Natori (1985). *Biochem. J.* 229, 453; Reddy and Bhargava, (1979). *Nature* 279, 725; Reichhart et al., (1989). *Eur. J. Biochem.* 182, 423; Romeo et al., (1988). *JBC* 263, 9573; Samakovlis et al., (1991). *EMBO J.* 10, 163; Schmidt et al., (1992). *Toxicon* 30, 1027; Schweitz et al., (1989). *Biochem.* 28, 9708; Selsted et al., (1983). *JBC* 258, 14485; Selsted et al., (1992). *JBC* 267, 4292; Simmaco et al., (1993). *FEBS Letters* 324, 159; Sures and Crippa (1984). *PNAS* 81, 380; Takada et al., (1984). *Infect. and Imm.* 44, 370; Tosteson and Tosteson, (1984). *Biophysical J.* 45, 112; Tryselius et al., (1992). *Eur. J. Biochem.* 204, 395; Xanthopoulos et al., (1988). *Eur. J. Biochem.* 172, 371; Yamashita and Saito, (1989). *Infect. and Imm.* 57, 2405; Zasloff, M. (1987). *PNAS* 84, 5449.

In addition to the peptides listed above, chimeras and analogues of these peptides are useful within the context of the present invention. For this invention, analogues of native cationic peptides must retain a net positive charge, but may contain D-amino acids, amino acid derivatives, insertions, deletions, and the like, some of which are discussed below. Chimeras include fusions of cationic peptide, such as the peptides of fragments thereof listed above, and fusions of cationic peptides with non-cationic peptides.

As described herein, modification of any of the residues including the N- or C-terminus is within the scope of the invention. A preferred modification of the C-terminus is amidation. Other modifications of the C-terminus include esterification and lactone formation. N-terminal modifications include acetylation, acylation, alkylation, PEGylation, myristylation, and the like. Additionally, the peptide may be modified to form an polymer-modified peptide as described below. The peptides may also be labeled, such as with a radioactive label, a fluorescent label, a mass spectrometry tag, biotin and the like.

A. Indolicidin and Analogues

As used herein, "indolicidin" refers to an antimicrobial cationic peptide. Indolicidins may be isolated from a variety of organisms. One indolicidin is isolated from bovine neutrophils and is a 13 amino acid peptide amidated at the carboxy-terminus in its native form (Selsted et al., *J. Biol. Chem. 267*:4292, 1992). An amino acid sequence of indolicidin is presented in SEQ ID NO: 1.

B. Cecropin peptides

Cecropins are cationic peptides that have antimicrobial activity against both Gram-positive and Gram-negative bacteria. Cecropins have been isolated from both invertebrates (*e.g.*, insect hemolymph) as well as vertebrates (*e.g.* pig intestines). Generally, these peptides are 35 to 39 residues. An exemplary cecropin has the sequence KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK (SEQ ID No. ___). Some additional cecropin sequences are presented in Table 1. Within the context of this invention, cecropins include analogues that have one or more insertions, deletions, modified amino acids, D-amino acids and the like.

C. Melittin peptides

Melittin is a cationic peptide found in bee venom. An amino acid sequence of an exemplary melittin peptide is GIGAVLKVLTTGLPALISWIKRKKRQQ (SEQ ID No. ___). Like the cecropins, melittin exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria. Within the context of this invention, melittin includes analogues that have one or more insertions, deletions, modified amino acids, D-amino acids and the like.

D. Cecropin-melittin chimeric peptides

As noted herein, cationic peptides include fusion peptides of native cationic peptides and analogues of fusion peptides. In particular, fusions of cecropin and melittin are provided. An exemplary fusion has the sequence: cecropin A (residues 1-8)/melittin (residues 1-18). Other fusion peptides useful within the context of this invention are described by the general formulas below.

K W K R_2 R_1 R_1 R_2 R_2 R_1 R_2 R_2 R_1 R_1 R_2 R_2 V L T T G L P A L I S (SEQ ID No. ____)
K W K R_2 R_1 R_1 R_2 R_2 R_1 R_2 R_2 R_1 R_1 R_2 R_2 V V T T A K P L I S S (SEQ ID No. ____)
K W K R_2 R_1 R_1 R_2 R_2 R_1 R_2 R_2 R_1 R_1 R_2 R_2 I L T T G L P A L I S (SEQ ID No. ____)
K W K R_2 R_1 R_1 R_2 R_2 R_1 R_2 R_2 R_1 R_1 R_2 R_2 G G L L S N I V T S L (SEQ ID No. ____)
K W K R_2 R_1 R_1 R_2 R_2 R_1 R_2 R_2 R_1 R_1 R_2 R_2 G P I L A N L V S I V (SEQ ID No. ____)
K K W W R R R_1 R_1 R_2 R_1 R_1 R_2 R_2 G P A L S N V (SEQ ID No. ____)
K K W W R R X (SEQ ID No. ____)
K K W W K X (SEQ ID No. ____)

wherein R_1 is a hydrophobic amino acid residue, R_2 is a hydrophilic amino acid residue, and X is from about 14 to 24 amino acid residues.

E. Drosocin and analogues

As noted herein, cationic peptides include drosocin and drosocin analogues. Drosocins are isolated from *Drosophila melanogaster*. An exemplary drosocin is a 19 amino acid peptide having the sequence: GKPRPYSPRPTSHPRPIRV (SEQ ID No. ____; GenBank

Accession No. S35984). Analogues of drosocin include peptides that have insertions, deletions, modified amino acids, D-amino acids and the like.

F. Peptide synthesis

Peptides may be synthesized by standard chemical methods, including synthesis by automated procedure. In general, peptide analogues are synthesized based on the standard solid-phase Fmoc protection strategy with HATU as the coupling agent. The peptide is cleaved from the solid-phase resin with trifluoroacetic acid containing appropriate scavengers, which also deprotects side chain functional groups. Crude peptide is further purified using preparative reversed-phase chromatography. Other purification methods, such as partition chromatography, gel filtration, gel electrophoresis, or ion-exchange chromatography may be used.

Other synthesis techniques, known in the art, such as the tBoc protection strategy, or use of different coupling reagents or the like can be employed to produce equivalent peptides. Peptides may be synthesized as a linear molecule or as branched molecules. Branched peptides typically contain a core peptide that provides a number of attachment points for additional peptides. Lysine is most commonly used for the core peptide because it has one carboxyl functional group and two (alpha and epsilon) amine functional groups. Other diamino acids can also be used. To synthesize these multimeric peptides, the solid phase resin is derivatized with the core matrix, and subsequent synthesis and cleavage from the resin follows standard procedures. The multimeric peptides may be used within the context of this invention as for any of the linear peptides.

G. Recombinant production of peptides

Peptides may alternatively be synthesized by recombinant production (*see e.g.*, U.S. Patent No. 5,593,866). A variety of host systems are suitable for production of the peptide analogues, including bacteria (*e.g.*, *E. coli*), yeast (*e.g.*, *Saccharomyces cerevisiae*), insect (*e.g.*, Sf9), and mammalian cells (*e.g.*, CHO, COS-7). Many expression vectors have been developed and are available for each of these hosts. Generally, bacteria cells and vectors that are functional in bacteria are used in this invention. However, at times, it may be preferable to have vectors that are functional in other hosts. Vectors and procedures for

cloning and expression in *E. coli* are discussed herein and, for example, in Sambrook et al. (*Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1987) and in Ausubel et al. (*Current Protocols in Molecular Biology,* Greene Publishing Co., 1995).

A DNA sequence encoding a cationic peptide is introduced into an expression vector appropriate for the host. In preferred embodiments, the gene is cloned into a vector to create a fusion protein. The fusion partner is chosen to contain an anionic region, such that a bacterial host is protected from the toxic effect of the peptide. This protective region effectively neutralizes the antimicrobial effects of the peptide and also may prevent peptide degradation by host proteases. The fusion partner (carrier protein) of the invention may further function to transport the fusion peptide to inclusion bodies, the periplasm, the outer membrane, or the extracellular environment. Carrier proteins suitable in the context of this invention specifically include, but are not limited to, glutathione-S-transferase (GST), protein A from *Staphylococcus aureus*, two synthetic IgG-binding domains (ZZ) of protein A, outer membrane protein F, β-galactosidase (*lacZ*), and various products of bacteriophage λ and bacteriophage T7. Furthermore, the entire carrier protein need not be used, as long as the protective anionic region is present.

To facilitate isolation of the peptide sequence, amino acids susceptible to chemical cleavage (*e.g.*, CNBr) or enzymatic cleavage (*e.g.*, V8 protease, trypsin) are used to bridge the peptide and fusion partner. For expression in *E. coli*, the fusion partner is preferably a normal intracellular protein that directs expression toward inclusion body formation. In such a case, following cleavage to release the final product, there is no requirement for renaturation of the peptide.

In the present invention, the DNA cassette, comprising fusion partner and peptide gene, may be inserted into an expression vector, which can be a plasmid, virus or other vehicle known in the art. At minimum, the expression vector should contain a promoter sequence. However, other regulatory sequences may also be included. Such sequences include an enhancer, ribosome binding site, transcription termination signal sequence, secretion signal sequence, origin of replication, selectable marker, and the like. The regulatory sequences are operationally associated with one another to allow transcription and subsequent translation. Preferably, the expression vector is a plasmid that contains an inducible or constitutive promoter to facilitate the efficient transcription of the inserted DNA

sequence in the host. Transformation of the host cell with the recombinant DNA may be carried out by Ca^{++}-mediated techniques, by electroporation, or other methods well known to those skilled in the art.

The peptide product is isolated by standard techniques, such as affinity, size exclusion, or ionic exchange chromatography, HPLC and the like. An isolated peptide should preferably show a major band by Coomassie blue stain of SDS-PAGE that is at least 90% of the material.

II. TESTING

Cationic peptides of the present invention are assessed either alone or in combination with an antibiotic agent or another analogue for their potential as antibiotic therapeutic agents using a series of assays. Preferably, all peptides are initially assessed *in vitro*, the most promising candidates are selected for further assessment *in vivo*, and then candidates are selected for pre-clinical studies. *In vitro* assays include measurement of antibiotic activity, toxicity, solubility, pharmacology, secondary structure, liposome permeabilization and the like. *In vivo* assays include assessment of efficacy in animal models, antigenicity, toxicity, and the like. In general, *in vitro* assays are initially performed, followed by *in vivo* assays.

Peptides that have some anti-microbial activity are preferred, although such activity may not be necessary for enhancing the activity of an antibiotic agent. Also, for *in vivo* use, peptides should preferably demonstrate acceptable toxicity profiles, as measured by standard procedures. Lower toxicity is preferred..

A. *In vitro* assays

Cationic peptides, including indolicidin analogues, are assayed by, for example, an agarose dilution MIC assay, a broth dilution assay, time-kill assay, or equivalent methods. Antibiotic activity is measured as inhibition of growth or killing of a microorganism (*e.g.*, bacteria, fungi).

Briefly, a candidate peptide in Mueller Hinton broth supplemented with calcium and magnesium is mixed with molten agarose. Other broths and agars may be used as long as the peptide can freely diffuse through the medium. The agarose is poured into petri

dishes or wells, allowed to solidify, and a test strain is applied to the agarose plate. The test strain is chosen, in part, on the intended application of the peptide. Thus, by way of example, if an indolicidin analogue with activity against *S. aureus* is desired, an *S. aureus* strain is used. It may be desirable to assay the analogue on several strains and/or on clinical isolates of the test species. Plates are incubated overnight and inspected visually for bacterial growth. A minimum inhibitory concentration (MIC) of a cationic peptide is the lowest concentration of peptide that completely inhibits growth of the organism. Peptides that exhibit good activity against the test strain, or group of strains, typically having an MIC of less than or equal to 16 μg/ml are selected for further testing.

Alternatively, time kill curves can be used to determine the differences in colony counts over a set time period, typically 24 hours. Briefly, a suspension of organisms of known concentration is prepared and a candidate peptide is added. Aliquots of the suspension are removed at set times, diluted, plated on medium, incubated, and counted. MIC is measured as the lowest concentration of peptide that completely inhibits growth of the organism. In general, lower MIC values are preferred.

Candidate cationic peptides may be further tested for their toxicity to normal mammalian cells. An exemplary assay is a red blood cell (RBC) (erythrocyte) hemolysis assay. Briefly, in this assay, red blood cells are isolated from whole blood, typically by centrifugation, and washed free of plasma components. A 5% (v/v) suspension of erythrocytes in isotonic saline is incubated with different concentrations of peptide analogue. Generally, the peptide will be in a suitable formulation buffer. After incubation for approximately 1 hour at 37°C, the cells are centrifuged, and the absorbance of the supernatant at 540 nm is determined. A relative measure of lysis is determined by comparison to absorbance after complete lysis of erythrocytes using NH_4Cl or equivalent (establishing a 100% value). A peptide with <10% lysis at 100 μg/ml is suitable. Preferably, there is <5% lysis at 100 μg/ml. Such peptides that are not lytic, or are only moderately lytic, are desirable and suitable for further screening. Other *in vitro* toxicity assays, for example measurement of toxicity towards cultured mammalian cells, may be used to assess *in vitro* toxicity.

Solubility of the peptide in formulation buffer is an additional parameter that may be examined. Several different assays may be used, such as appearance in buffer. Briefly, peptide is suspended in solution, such as broth or formulation buffer. The appearance is evaluated according to a scale that ranges from (a) clear, no precipitate, (b)

light, diffuse precipitate, to (c) cloudy, heavy precipitate. Finer gradations may be used. In general, less precipitate is more desirable. However, some precipitate may be acceptable.

Additional *in vitro* assays may be carried out to assess the potential of the peptide as a therapeutic. Such assays include peptide solubility in formulations, pharmacology in blood or plasma, serum protein binding, analysis of secondary structure, for example by circular dichroism, liposome permeabilization, and bacterial inner membrane permeabilization.

B. *In vivo* assays

Peptides, including peptide analogues, selected on the basis of the results from the in vitro assays can be tested *in vivo* for efficacy, toxicity and the like.

The antibiotic activity of selected peptides may be assessed *in vivo* for their ability to ameliorate microbial infections using animal models. A variety of methods and animal models are available. Within these assays, a peptide is useful as a therapeutic if inhibition of microorganismal growth compared to inhibition with vehicle alone is statistically significant. This measurement can be made directly from cultures isolated from body fluids or sites, or indirectly, by assessing survival rates of infected animals. For assessment of antibacterial activity several animal models are available, such as acute infection models including those in which (a) normal mice receive a lethal dose of microorganisms, (b) neutropenic mice receive a lethal dose of microorganisms or (c) rabbits receive an inoculum in the heart, and chronic infection models. The model selected will depend in part on the intended clinical indication of the analogue.

By way of example, in a normal mouse model, mice are inoculated ip or iv with a lethal dose of bacteria. Typically, the dose is such that 90-100% of animals die within 2 days. The choice of a microorganismal strain for this assay depends, in part, upon the intended application of the analogue, and in the accompanying examples, assays are carried out with three different *Staphylococcus* strains. Briefly, shortly before or after inoculation (generally within 60 minutes), analogue in a suitable formulation buffer is injected. Multiple injections of analogue may be administered. Animals are observed for up to 8 days post-infection and the survival of animals is recorded. Successful treatment either rescues animals from death or delays death to a statistically significant level, as compared with non-treatment control animals

In vivo toxicity of a peptide is measured through administration of a range of doses to animals, typically mice, by a route defined in part by the intended clinical use. The survival of the animals is recorded and LD_{50}, $LD_{90\text{-}100}$, and maximum tolerated dose (MTD) can be calculated to enable comparison of analogues.

Furthermore, for *in vivo* use, low immunogenicity is preferred. To measure immunogenicity, peptides are injected into normal animals, generally rabbits. At various times after a single or multiple injections, serum is obtained and tested for antibody reactivity to the peptide analogue. Antibodies to peptides may be identified by ELISA, immunoprecipitation assays, Western blots, and other methods. (*see*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988). No or minimal antibody reactivity is preferred. Additionally, pharmacokinetics of the analogues in animals and histopathology of animals treated with analogues may be determined.

Selection of cationic peptides as potential therapeutics is based on *in vitro* and *in vivo* assay results. In general, peptides that exhibit low toxicity at high dose levels and high efficacy at low dose levels are preferred candidates.

III. ANTIBIOTIC AGENTS

An antibiotic agent includes any molecule that tends to prevent, inhibit or destroy life and as such, includes anti-bacterial agents, anti-fungicides, anti-viral agents, and anti-parasitic agents. These agents may be isolated from an organism that produces the agent or procured from a commercial source (*e.g.*, pharmaceutical company, such as Eli Lilly, Indianapolis, IN; Sigma, St. Louis, MO).

Anti-bacterial antibiotic agents include, but are not limited to, penicillins, cephalosporins, carbacephems, cephamycins, carbapenems, monobactams, aminoglycosides, glycopeptides, quinolones, tetracyclines, macrolides, and fluoroquinolones Examples of antibiotic agents include, but are not limited to, Penicillin G (CAS Registry No.: 61-33-6); Methicillin (CAS Registry No.: 61-32-5); Nafcillin (CAS Registry No.: 147-52-4); Oxacillin (CAS Registry No.: 66-79-5); Cloxacillin (CAS Registry No.: 61-72-3); Dicloxacillin (CAS Registry No.: 3116-76-5); Ampicillin (CAS Registry No.: 69-53-4); Amoxicillin (CAS Registry No.: 26787-78-0); Ticarcillin (CAS Registry No.: 34787-01-4); Carbenicillin (CAS

Registry No.: 4697-36-3); Mezlocillin (CAS Registry No.: 51481-65-3); Azlocillin (CAS Registry No.: 37091-66-0); Piperacillin (CAS Registry No.: 61477-96-1); Imipenem (CAS Registry No.: 74431-23-5); Aztreonam (CAS Registry No.: 78110-38-0); Cephalothin (CAS Registry No.: 153-61-7); Cefazolin (CAS Registry No.: 25953-19-9); Cefaclor (CAS Registry No.: 70356-03-5); Cefamandole formate sodium (CAS Registry No.: 42540-40-9); Cefoxitin (CAS Registry No.: 35607-66-0); Cefuroxime (CAS Registry No.: 55268-75-2); Cefonicid (CAS Registry No.: 61270-58-4); Cefmetazole (CAS Registry No.: 56796-20-4); Cefotetan (CAS Registry No.: 69712-56-7); Cefprozil (CAS Registry No.: 92665-29-7); Loracarbef (CAS Registry No.: 121961-22-6); Cefetamet (CAS Registry No.: 65052-63-3); Cefoperazone (CAS Registry No.: 62893-19-0); Cefotaxime (CAS Registry No.: 63527-52-6); Ceftizoxime (CAS Registry No.: 68401-81-0); Ceftriaxone (CAS Registry No.: 73384-59-5); Ceftazidime (CAS Registry No.: 72558-82-8); Cefepime (CAS Registry No.: 88040-23-7); Cefixime (CAS Registry No.: 79350-37-1); Cefpodoxime (CAS Registry No.: 80210-62-4); Cefsulodin (CAS Registry No.: 62587-73-9); Fleroxacin (CAS Registry No.: 79660-72-3); Nalidixic acid (CAS Registry No.: 389-08-2); Norfloxacin (CAS Registry No.: 70458-96-7); Ciprofloxacin (CAS Registry No.: 85721-33-1); Ofloxacin (CAS Registry No.: 82419-36-1); Enoxacin (CAS Registry No.: 74011-58-8); Lomefloxacin (CAS Registry No.: 98079-51-7); Cinoxacin (CAS Registry No.: 28657-80-9); Doxycycline (CAS Registry No.: 564-25-0); Minocycline (CAS Registry No.: 10118-90-8); Tetracycline (CAS Registry No.: 60-54-8); Amikacin (CAS Registry No.: 37517-28-5); Gentamicin (CAS Registry No.: 1403-66-3); Kanamycin (CAS Registry No.: 8063-07-8); Netilmicin (CAS Registry No.: 56391-56-1); Tobramycin (CAS Registry No.: 32986-56-4); Streptomycin (CAS Registry No.: 57-92-1); Azithromycin (CAS Registry No.: 83905-01-5); Clarithromycin (CAS Registry No.: 81103-11-9); Erythromycin (CAS Registry No.: 114-07-8); Erythromycin estolate (CAS Registry No.: 3521-62-8); Erythromycin ethyl succinate (CAS Registry No.: 41342-53-4); Erythromycin glucoheptonate (CAS Registry No.: 23067-13-2); Erythromycin lactobionate (CAS Registry No.: 3847-29-8); Erythromycin stearate (CAS Registry No.: 643-22-1); Vancomycin (CAS Registry No.: 1404-90-6); Teicoplanin (CAS Registry No.: 61036-64-4); Chloramphenicol (CAS Registry No.: 56-75-7); Clindamycin (CAS Registry No.: 18323-44-9); Trimethoprim (CAS Registry No.: 738-70-5); Sulfamethoxazole (CAS Registry No.: 723-46-6); Nitrofurantoin (CAS Registry No.: 67-20-9); Rifampin (CAS Registry No.: 13292-46-1); Mupirocin (CAS Registry No.: 12650-69-0); Metronidazole (CAS Registry No.: 443-48-

1); Cephalexin (CAS Registry No.: 15686-71-2); Roxithromycin (CAS Registry No.: 80214-83-1); Co-amoxiclavuanate; combinations of Piperacillin and Tazobactam; and their various salts, acids, bases, and other derivatives.

Table 2 presents categories of antibiotics, their mode of action and examples of antibiotics.

Table 2

Class of Antibiotic	Antibiotic	Mode of Action
PENICILLINS		Blocks the formation of new cell walls in bacteria
Natural	Penicillin G, Benzylpenicillin Penicillin V, Phenoxymethylpenicillin	
Penicillinase resistant	Methicillin, Nafcillin, Oxacillin Cloxacillin, Dicloxacillin	
Acylamino-penicillins	Ampicillin, Amoxicillin	
Carboxy-penicillins	Ticarcillin, Carbenicillin	
Ureido-penicillins	Mezlocillin, Azlocillin, Piperacillin	
CARBAPENEMS	Imipenem, Meropenem	Blocks the formation of new cell walls in bacteria
MONOBACTAMS	Aztreonam	Blocks the formation of new cell walls in bacteria
CEPHALOSPORINS		Prevents formation of new cell walls in bacteria
1st Generation	Cephalothin, Cefazolin	
2nd Generation	Cefaclor, Cefamandole Cefuroxime, Cefonicid, Cefmetazole, Cefotetan, Cefprozil	
3rd Generation	Cefetamet, Cefoperazone Cefotaxime, Ceftizoxime Ceftriaxone, Ceftazidime Cefixime, Cefpodoxime, Cefsulodin	
4th Generation	Cefepime	
CARBACEPHEMS	Loracarbef	Prevents formation of new cell walls in bacteria
CEPHAMYCINS	Cefoxitin	Prevents formation of new cell walls in bacteria
QUINOLONES	Fleroxacin, Nalidixic Acid Norfloxacin, Ciprofloxacin Ofloxacin, Enoxacin Lomefloxacin, Cinoxacin	Inhibits bacterial DNA synthesis

Class of Antibiotic	Antibiotic	Mode of Action
TETRACYCLINES	Doxycycline, Minocycline, Tetracycline	Inhibits bacterial protein synthesis, binds to 30S ribosome subunit.
AMINOGLYCOSIDES	Amikacin, Gentamicin, Kanamycin, Netilmicin, Tobramycin, Streptomycin	Inhibits bacterial protein synthesis, binds to 30S ribosome subunit.
MACROLIDES	Azithromycin, Clarithromycin, Erythromycin	Inhibits bacterial protein synthesis, binds to 50S ribosome subunit
Derivatives of Erythromycin	Erythromycin estolate, Erythromycin stearate Erythromycin ethylsuccinate Erythromycin gluceptate Erythromycin lactobionate	
GLYCOPEPTIDES	Vancomycin, Teicoplanin	Inhibits cell wall synthesis, prevents peptidoglycan elongation.
MISCELLANEOUS	Chloramphenicol	Inhibits bacterial protein synthesis, binds to 50S ribosome subunit.
	Clindamycin	Inhibits bacterial protein synthesis, binds to 50S ribosome subunit.
	Trimethoprim	Inhibits the enzyme dihydrofolate reductase, which activates folic acid.
	Sulfamethoxazole	Acts as antimetabolite of PABA & inhibits synthesis of folic acid
	Nitrofurantoin	Action unknown, but is concentrated in urine where it can act on urinary tract bacteria
	Rifampin	Inhibits bacterial RNA polymerase
	Mupirocin	Inhibits bacterial protein synthesis

Anti-fungal agents include, but are not limited to, terbinafine hydrochloride, nystatin, amphotericin B, griseofulvin, ketoconazole, miconazole nitrate, flucytosine, fluconazole, itraconazole, clotrimazole, benzoic acid, salicylic acid, and selenium sulfide.

Anti-viral agents include, but are not limited to, amantadine hydrochloride, rimantadin, acyclovir, famciclovir, foscarnet, ganciclovir sodium, idoxuridine, ribavirin, sorivudine, trifluridine, valacyclovir, vidarabin, didanosine, stavudine, zalcitabine, zidovudine, interferon alpha, and edoxudine.

Anti-parasitic agents include, but are not limited to, pirethrins/piperonyl butoxide, permethrin, iodoquinol, metronidazole, diethylcarbamazine citrate, piperazine, pyrantel pamoate, mebendazole, thiabendazole, praziquantel, albendazole, proguanil, quinidine gluconate injection, quinine sulfate, chloroquine phosphate, mefloquine hydrochloride, primaquine phosphate, atovaquone, co-trimoxazole (sulfamethoxazole/trimethoprim), and pentamidine isethionate.

IV. ENHANCED ACTIVITY OF COMBINATIONS OF CATIONIC PEPTIDES AND ANTIBIOTIC AGENTS

Enhanced activity occurs when a combination of peptide and antibiotic agent potentiates activity beyond the individual effects of the peptide or antibiotic agent alone or additive effects of peptide plus antibiotic agent. Enhanced activity is especially desirable in at least four scenarios: (1) the microorganism is sensitive to the antibiotic agent, but the dosage has associated problems; (2) the microorganism is tolerant to the antibiotic agent, and is inhibited from growing but is not killed; (3) the microorganism is inherently resistant to the antibiotic agent; and (4) the microorganism has acquired resistance to the antibiotic agent. Enhanced efficacy resulting from administration of the antibiotic agent in combination with a cationic peptide in the above scenarios: (1) allows for administration of lower dosages ofr antibiotic agent or cationic peptide; (2) restores a cytocidal effect; (3) overcomes inherent resistance; and (4) overcomes acquired resistance.

A. Enhancement of antibiotic agent or cationic peptide activity

A synergistic combination of cationic peptide and antibiotic agent may permit a reduction in the dosage of one or both agents in order to achieve a similar therapeutic effect. This would allow smaller doses to be used, thus, decreasing the incidence of toxicity (*e.g.*, from aminoglycosides) and lowering costs of expensive antibiotics (*e.g.*, vancomycin). Concurrent or sequential administration of peptide and antibiotic agent is expected to provide more effective treatment of infections caused by micro-organisms (bacteria, viruses, fungi, and parasites). In particular, this could be achieved by using doses of the peptide or antibiotic agent alone would not achieve therapeutic success. Alternatively, the antibiotic agent and peptide can be administered at therapeutic doses for each, but wherein the combination of the two agents provides even more potent effects.

As used herein, "synergy" refers to the *in vitro* effect of administration of a combination of a cationic peptide and antibiotic agent such that (1) the fractional inhibitory concentration (FIC) is less than or equal to 0.5 in an FIC assay described herein; or (2) there is at least a 100-fold ($2\log_{10}$) increase in killing at 24 hours for the combination as compared with the antibiotic agent alone in a time kill curve assay as described herein.

Such synergy is conveniently measured in an *in vitro* assay, such as kinetic kill studies or a fractional inhibitory concentration (FIC) assay as determined by agarose or broth dilution assay. The agarose dilution assay is preferred.

Briefly, in the dilution assay, a checkerboard array of cationic peptides and antibiotic agents titrated in doubling dilutions are inoculated with a microbial (*e.g.*, bacterial) isolate. The FIC is determined by observing the impact of one antibiotic agent on the MIC ("minimal inhibitory concentration") of the cationic peptide and vice versa. FIC is calculated by the following formula:

$$FIC = \frac{MIC(peptide\ in\ combination)}{MIC(peptide\ alone)} + \frac{MIC(antibiotic\ in\ combination)}{MIC(antibiotic\ alone)}$$

An FIC of ≤ 0.5 is evidence of synergy. An additive response has an FIC value of > 0.5 and less than or equal to 1, while an indifferent response has an FIC value of >1 and ≤ 2. Although a synergistic effect is preferred, an additive effect may still indicate that the combination of antibiotic agent and cationic peptide are therapeutically useful.

B. Overcoming tolerance

Tolerance is associated with a defect in bacterial cellular autolytic enzymes such that an antibacterial agent demonstrates bacteriostatic rather than bactericidal activity (Mahon and Manuselis, *Textbook of Diagnostic Microbiology*, W.B. Saunders Co., Toronto, Canada, p. 92, 1995). For antibiotic agents that have only bacteriostatic activity, the administration of cationic peptides in combination with antibiotic agents can restore bactericidal activity. Alternatively, the addition of a peptide to an antibiotic agent may increase the rate of a bactericidal effect of an antibiotic.

Bactericidal effects of antibiotics can be measured *in vitro* by a variety of assays. Typically, the assay is a measurement of MBC ("minimal bactericidal concentration"), which is an extension of the MIC determination. The agarose dilution assay is adapted to provide both MBC and MIC for an antimicrobial agent alone and the agent in combination with a cationic peptide. Alternatively, kinetic time-kill (or growth) curves can be used to determine MIC and MBC.

Briefly, following determination of MIC, MBC is determined from the assay plates by swabbing the inocula on plates containing antibiotic agent in concentrations at and above the MIC, resuspending the swab in saline or medium, and plating an aliquot on agarose plates. If the number of colonies on these agarose plates is less than 0.1% of the initial inoculum (as determined by a plate count immediately after inoculation of the MIC test plates), then ≥ 99.9% killing has occurred. The MBC end point is defined as the lowest concentration of the antimicrobial agent that kills 99.9% of the test bacteria.

Thus, tolerance of a microorganism to an antimicrobial agent is indicated when the number of colonies growing on subculture plates exceeds the 0.1% cutoff for several successive concentrations above the observed MIC. A combination of antimicrobial agent and cationic peptide that breaks tolerance results in a decrease in the MBC:MIC ratio to < 32.

C. Overcoming inherent resistance

The combination of a cationic peptide with an antibiotic agent, for which a microorganism is inherently resistant (*i.e.*, the antibiotic has never been shown to be

therapeutically effective against the organism in question), is used to overcome the resistance and confer susceptibility of the microorganism to the agent. Overcoming inherent resistance is especially useful for infections where the causative organism is becoming or has become resistant to most, if not all, of the currently prescribed antibiotics. Additionally, administering a combination therapy provides more options when toxicity of an antibiotic agent and/or price are a consideration.

Overcoming resistance can be conveniently measured *in vitro*. Resistance is overcome when the MIC for a particular antibiotic agent against a particular microorganism is decreased from the resistant range to the sensitive range (according to the National Committee for Clinical Laboratory Standards (NCCLS)) (*see also*, Moellering, in *Principles and Practice of Infectious Diseases*, 4th edition, Mandell et al., eds. Churchill Livingstone, NY, 1995). NCCLS standards are based on microbiological data in relation to pharmacokinetic data and clinical studies. Resistance is determined when the organism causing the infection is not inhibited by the normal achievable serum concentrations of the antibiotic agent based on recommended dosage. Susceptibility is determined when the organism responds to therapy with the antibiotic agent used at the recommended dosage for the type of infection and microorganism.

D. Overcoming acquired resistance

Acquired resistance in a microorganism that was previously sensitive to an antibiotic agent is generally due to mutational events in chromosomal DNA, acquisition of a resistance factor carried via plasmids or phage, or transposition of a resistance gene or genes from a plasmid or phage to chromosomal DNA.

When a microorganism acquires resistance to an antibiotic, the combination of a peptide and antibiotic agent can restore activity of the antibiotic agent by overcoming the resistance mechanism of the organism. This is particularly useful for organisms that are difficult to treat or where current therapy is costly or toxic. The ability to use a less expensive or less toxic antibiotic agent, which had been effective in the past, is an improvement for certain current therapies. The re-introduction of an antibiotic agent would enable previous clinical studies and prescription data to be used in its evaluation. Activity is measured *in vitro* by MICs or kinetic kill curves and *in vivo* using animal and human clinical trials.

E. Enhancement of effect of lysozyme and nisin

The combination of lysozyme or nisin with an antibiotic may improve their antibacterial effectiveness and allow use in situations in which the single agent is inactive or inappropriate.

Lysozymes disrupt certain bacteria by cleaving the glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide component of bacterial cell walls. However, lysozyme exhibits only weak antibacterial activity with a narrow spectrum of activity. The addition of an antibiotic may improve the effectiveness of this activity and broaden the spectrum of activity.

Nisins are 34-residue peptide lantibiotics with primarily anti-Gram-positive bacterial activity. Nisin is used in the food processing industry as a preservative, especially for cheese, canned fruits and vegetables. Nisin forms transient potential-dependent pores in the bacterial cytoplasmic membranes but also exhibits weak antibacterial activity with a narrow spectrum of activity. The addition of an antibiotic may improve the effectiveness of nisin and broaden the spectrum of activity.

F. *In vivo* assays

In vivo testing involves the use of animal models of infection. Typically, but not exclusively, mice are used. The test organism is chosen according to the intended combination of cationic peptide and antibiotic to be evaluated. Generally, the test organism is injected intraperitoneally (IP) or intravenously (IV) at 10 to 100 times the fifty percent lethal dose (LD_{50}). The LD_{50} is calculated using a method described by Reed and Muench (Reed LJ and Muench H. *The American Journal of Hygiene, 27*:493-7.). The antibiotic agent and the cationic peptide are injected IP, IV, or subcutaneously (SC) individually as well as in combination to different groups of mice. The antimicrobial agents may be given in one or multiple doses. Animals are observed for 5 to 7 days. Other models of infection may also be used according to the clinical indication for the combination of antibiotic agents.

The number of mice in each group that survive the infectious insult is determined after 5 to 7 days. In addition, when bacteria are the test organisms, bacterial colony counts from blood, peritoneal lavage fluid, fluid from other body sites, and/or tissue

from different body sites taken at various time intervals can be used to assess efficacy. Samples are serially diluted in isotonic saline and incubated for 20 - 24 hours, at 37° C, on a suitable growth medium for the bacterium.

Synergy between the cationic peptide and the antibiotic agent is assessed using a model of infection as described above. For a determination of synergy, one or more of the following should occur. The combination group should show greater survival rates compared to the groups treated with only one agent; the combination group and the antibiotic agent group have equivalent survival rates with the combination group receiving a lower concentration of antibiotic agent; the combination group has equivalent or better survival compared to an antibiotic agent group with a lower microorganismal load at various time points.

Overcoming tolerance can be demonstrated by lower bacterial colony counts at various time points in the combination group over the antibiotic agent group. This may also result in better survival rates for the combination group.

Similar animal models to those described above can be used to establish when inherent or acquired resistance is overcome. The microorganism strain used is, by definition, resistant to the antibiotic agent and so the survival rate in the antibiotic agent group will be close, if not equal, to zero percent. Thus, overcoming the inherent resistance of the microorganism to the antibiotic agent is demonstrated by increased survival of the combination group. Testing for reversing acquired resistance may be performed in a similar manner.

V. COMBINATIONS OF PEPTIDES AND ANTIBIOTIC AGENTS

As discussed herein, cationic peptides are administered in combination with antibiotic agents. The combination enhances the activity of the antibiotic agents. Such combinations may be used to effect a synergistic result, overcome tolerance, overcome inherent resistance, or overcome acquired resistance of the microorganism to the antibiotic agent.

To achieve a synergistic effect, a combination of antibiotic agent and cationic peptide is administered to a patient or administered in such a manner as to contact the microorganism. Any combination of antibiotic agent and cationic peptide may result in a synergistic effect and, thus, is useful within the context of this invention.

In particular, certain microorganisms are preferred targets. In conjunction with these microorganisms, certain commonly used antibiotic agents are preferred to be enhanced. The table below sets out these microorganisms, antibiotic agents, and cationic peptide combinations that are preferred.

Table 3

BACTERIAL SPECIES	ANTIMICROBIAL AGENTS	PEPTIDE
A. baumannii	Gentamicin	MBI 21A2
B. cepacia	Ceftriaxone	MBI 11J02CN
E. cloacae	Ciprofloxacin	MBI 29A2
E. faecalis	Amikacin	MBI 11B16CN
E. faecium	Vancomycin	MBI 29
P. aeruginosa	Mupirocin	MBI 28
P. aeruginosa	Tobramycin	MBI 11G13CN
S marcescens	Pipcracillin	MBI 11G7CN
S. aureus	Piperacillin	MBI 11CN
S. maltophilia	Tobramycin	REWH 53A5CN
MYCOSES	**ANTIFUNGAL AGENTS**	**PEPTIDE**
Candida species	Fluconazole	MBI 28
Cryptococcus	Fluconazole	MBI 29A3
Aspergillus species	Itraconazole	MBI 26
VIRUSES	**ANTIVIRAL AGENTS**	**PEPTIDE**
Herpes simplex virus	Acyclovir	MBI 11A2CN
Influenza A	virus Amantadine-rimantadine	MBI 21A1
PARASITES	**ANTIPARASITIC AGENTS**	**PEPTIDE**
Trichomonas vaginalis	Metronidazole	MBI 29
Plasmodium falciparum	Chloroquine	MBI 11D18CN

To overcome tolerance, a combination of antibiotic agent and cationic peptide is administered to a patient or administered in such a manner as to contact the microorganism. Any combination of antibiotic agent and cationic peptide that overcomes tolerance is useful within the context of this invention. In particular, certain microorganisms, which exhibit tolerance to specific antibiotic agents are preferred targets. The table below sets out these microorganisms, antibiotic agents, and cationic peptide combinations that are preferred.

Table 4

BACTERIAL SPECIES	ANTIMICROBIAL AGENTS	PEPTIDE
Enterococcus species	Ampicillin (Amino-penicillins) Piperacillin (Penicillins, antipseudomonal)	MBI 21A10
Enterococcus species	Gentamicin (Aminoglycosides)	MBI 29
Enterococcus species	Vancomycin, Teicoplanin (glycopeptides)	MBI 26
Streptococcus pneumoniae	Penicillins	MBI 29A3
Salmonella typhi	Chloramphenicol	MBI 11A1CN
Campylobacter jejuni	Erythromycin (Macrolides)	MBI 11B4CN

To overcome inherent resistance, a combination of antibiotic agent and cationic peptide is administered to a patient or administered in such a manner as to contact the microorganism. Any combination of antibiotic agent and cationic peptide that overcomes resistance is useful within the context of this invention. In particular, certain microorganisms, which exhibit inherent resistance to specific antibiotic agents are preferred targets. The table below sets out these microorganisms, antibiotic agents, and cationic peptide combinations that are preferred.

Table 5

BACTERIAL SPECIES	ANTIMICROBIAL AGENTS	PEPTIDE
Methicillin-resistant *S. aureus*	Amikacin	MBI 29F1
S. maltophilia	Gentamicin	MBI 11D18CN
S. maltophilia	Gentamicin	MBI 26
S. maltophilia	Tobramycin	MBI 29A3
Methicillin-resistant *S. aureus*	Tobramycin	MBI 21A1
E. coli	Mupirocin	MBI 21A1
S. maltophilia	Amikacin	MBI 11B16CN
S. maltophilia	Amikacin	MBI 26
B. cepacia	Amikacin	MBI 29A3
Methicillin resistant *S. aureus*	Gentamicin	MBI 11D18CN
MYCOSES	**ANTIFUNGAL AGENTS**	**PEPTIDE**
Aspergillosis	Fluconazole	MBI 11D18CN
Candida species	Griseofulvin	MBI 29

To overcome acquired resistance, a combination of antibiotic agent and cationic peptide is administered to a patient or administered in such a manner as to contact the microorganism. Any combination of antibiotic agent and cationic peptide that overcomes resistance is useful within the context of this invention. In particular, certain microorganisms, which exhibit acquired resistance to specific antibiotic agents are preferred targets. The table

below sets out these microorganisms, antibiotic agents, and cationic peptide combinations that are preferred.

Table 6

BACTERIA	**ANTIMICROBIAL AGENT**	**PEPTIDE**
Enterococcus spp.	Vancomycin	MBI 26
P. aeruginosa	Ceftriaxone	MBI 26
S. aureus	Ciprofloxacin	MBI 29A2
E. cloacae	Piperacillin	MBI 11F4CN
P. aeruginosa	Tobramycin	MBI 21A1
P. aeruginosa	Ciprofloxacin	MBI 29A3
P. aeruginosa	Gentamicin	MBI 11B16CN
S. epidermidis	Gentamicin	MBI 11D18CN
Acinetobacter spp.	Tobramycin	MBI 11F3CN
Enterococcus spp.	Vancomycin	MBI 11A1CN
MYCOSES	**ANTIFUNGAL AGENTS**	**PEPTIDE**
Candida species	Fluconazole	MBI 11CN
Cryptococcus	Fluconazole	MBI 11A1CN
VIRUSES	**ANTIVIRAL AGENTS**	**PEPTIDE**
Herpes simplex virus	Acyclovir	MBI 29
Respiratory Syncytial Virus (RSV)	Ribavirin	MBI 26
Influenza A virus	Amantadine-rimantadine	MBI 26
PARASITES	**ANTIPARASITIC AGENTS**	**PEPTIDE**
Trichomonas vaginalis	Metronidazole	MBI 29
Pneumocystis carinii	Cotrimoxazole	MBI 29A3
Plasmodium falciparum	Chloroquine	MBI 26

Additional preferred combinations for indolicidin analogues are listed below:

ANTIBIOTIC	**PEPTIDE**
Ciprofloxacin	MBI 11A1CN
Vancomycin	MBI 11A1CN
Piperacillin	MBI 11B9CN
Gentamicin	MBI 11B16CN
Piperacillin	MBI 11D18CN
Tobramycin	MBI 11D18CN
Vancomycin	MBI 11D18CN
Piperacillin	MBI 11E3CN
Tobramycin	MBI 11F3CN
Piperacillin	MBI 11F4CN

VI. FORMULATIONS AND ADMINISTRATION

As noted above, the present invention provides methods for treating and preventing infections by administering to a patient a therapeutically effective amount of a

peptide analogue of indolicidin as described herein. Patients suitable for such treatment may be identified by well-established hallmarks of an infection, such as fever, pus, culture of organisms, and the like. Infections that may be treated with peptide analogues include those caused by or due to microorganisms. Examples of microorganisms include bacteria (*e.g.*, Gram-positive, Gram-negative), fungi, (*e.g.*, yeast and molds), parasites (*e.g.*, protozoans, nematodes, cestodes and trematodes), viruses, and prions. Specific organisms in these classes are well known (*see* for example, Davis et al., *Microbiology*, 3rd edition, Harper & Row, 1980). Infections include, but are not limited to, toxic shock syndrome, diphtheria, cholera, typhus, meningitis, whooping cough, botulism, tetanus, pyogenic infections, dysentery, gastroenteritis, anthrax, Lyme disease, syphilis, rubella, septicemia and plague.

More specifically, clinical indications include, but are not limited to: 1/ infections following insertion of intravascular devices or peritoneal dialysis catheters; 2/ infection associated with medical devices or prostheses; 3/ infection during hemodialysis; 4/ *S. aureus* nasal and extra-nasal carriage; 5/ burn wound infections; 6/ surgical wounds, 7/ acne, including severe acne vulgaris; 8/ nosocomial pneumonia; 9/ meningitis; 10/ cystic fibrosis; 11/ infective endocarditis; 12/ osteomyelitis; and 13/ sepsis in an immunocompromised host.

1/ Infections following insertion of contaminated intravascular devices, such as central venous catheters, or peritoneal dialysis catheters. These catheters are cuffed or non-cuffed, although the infection rate is higher for non-cuffed catheters. Both local and systemic infection may result from contaminated intravascular devices, more than 25,000 patients develop device related bacteremia in the United States each year. The main organisms responsible are coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, *Enterococcus* spp, *E. coli* and *Candida* spp.

The peptide and/or antibiotic, preferably as an ointment or cream, can be applied to the catheter site prior to insertion of the catheter and then again at each dressing change. The peptide may be incorporated into the ointment or cream at a concentration preferably of about 0.5 to about 2% (w/v).

2/ Infection associated with medical devices or prostheses, e.g. catheter, grafts, prosthetic heart valves, artificial joints, etc. One to five percent of indwelling prostheses become infected which usually requires removal or replacement of the prostheses. The main organisms responsible for these infections are CoNS and *S. aureus*.

Preferably, the peptide and/or antibiotic can be coated, either covalently bonded or by any other means, onto the medical device either at manufacture of the device or after manufacture but prior to insertion of the device. In such an application, the peptide antibiotic is preferably applied as a 0.5 to 2% solution.

3/ Infection during hemodialysis. Infection is the second leading cause of death in patients on chronic hemodialysis. Approximately 23% of bacteremias are due to access site infections. The majority of graft infections are caused by coagulate-positive (*S. aureus*) and coagulate-negative staphylococci. To combat infection, the peptide alone or in combination with an antibiotic can be applied as an ointment or cream to the dialysis site prior to each hemodialysis procedure.

4/ *S. aureus* nasal and extra-nasal carriage. Infection by this organism may result in impetigenous lesions or infected wounds. It is also associated with increased infection rates following cardiac surgery, hemodialysis, orthopedic surgery and neutropenia, both disease induced and iatrogenic. Nasal and extra-nasal carriage of staphylococci can result in hospital outbreaks of the same staphylococci strain that is colonizing a patient's or hospital worker's nasal passage or extra-nasal site. Much attention has been paid to the eradication of nasal colonization, but the results of treatment have been generally unsatisfactory. The use of topical antimicrobial substances, such as Bacitracin, Tetracycline, or Chlorhexidine, results in the suppression of nasal colonization, as opposed to its eradication.

The peptide alone or in combination with an antibiotic are preferably applied intra-nasally, formulated for nasal application, as a 0.5 to 2% ointment, cream or solution. Application may occur once or multiple times until the colonization of staphylococci is reduced or eliminated.

5/ Burn wound infections. Although the occurrence of invasive burn wound infections has been significantly reduced, infection remains the most common cause of morbidity and mortality in extensively burned patients. Infection is the predominant determinant of wound healing, incidence of complications, and outcome of burn patients. The main organisms responsible are *Pseudomonas aeruginosa, S. aureus, Streptococcus pyogenes*, and various gram-negative organisms. Frequent debridements and establishment of an epidermis, or a surrogate such as a graft or a skin substitute, is essential for prevention of infection.

The peptide alone or in combination with antibiotics can be applied to burn wounds as an ointment or cream and/or administered systemically. Topical application may prevent systemic infection following superficial colonization or eradicate a superficial infection. The peptide is preferably administered as a 0.5 to 2% cream or ointment. Application to the skin could be done once a day or as often as dressings are changed. The systemic administration could be by intravenous, intramuscular or subcutaneous injections or infusions. Other routes of administration could also be used.

6/ Surgical wounds, especially those associated with foreign material, e.g. sutures. As many as 71% of all nosocomial infections occur in surgical patients, 40% of which are infections at the operative site. Despite efforts to prevent infection, it is estimated that between 500,000 and 920,000 surgical wound infections complicate the approximately 23 million surgical procedures performed annually in the United States. The infecting organisms are varied but staphylococci are important organisms in these infections.

The peptide alone or with an antibiotic may be applied as an ointment, cream or liquid to the wound site or as a liquid in the wound prior to and during closure of the wound. Following closure the peptide antibiotic could be applied at dressing changes. For wounds that are infected, the peptide antibiotic could be applied topically and/or systemically.

7/ Acne, including severe acne vulgaris. This condition is due to colonization and infection of hair follicles and sebaceous cysts by *Propionibacterium acne*. Most cases remain mild and do not lead to scarring although a subset of patients develop large inflammatory cysts and nodules, which may drain and result in significant scarring.

The peptide alone or with an antibiotic can be incorporated into soap or applied topically as a cream, lotion or gel to the affected areas either once a day or multiple times during the day. The length of treatment may be for as long as the lesions are present or used to prevent recurrent lesions. The peptide antibiotic could also be administered orally or systemically to treat or prevent acne lesions.

8/ Nosocomial pneumonia. Nosocomial pneumonias account for nearly 20% of all nosocomial infections. Patients most at risk for developing nosocomial pneumonia are those in an intensive care unit, patients with altered levels of consciousness, elderly patients, patients with chronic lung disease, ventilated patients, smokers and post-operative patients. In a severely compromised patient, multiantibiotic-resistant nosocomial pathogens are likely to be the cause of the pneumonia.

The main organisms responsible are *P. aeruginosa*, *S. aureus*, *Klebsiella pneumoniae* and *Enterobacter* spp. The peptide alone or in combination with other antibiotics could be administered orally or systemically to treat pneumonia. Administration could be once a day or multiple administrations per day. Peptide antibiotics could be administered directly into the lung via inhalation or via installation of an endotracheal tube.

9/ Meningitis. Bacterial meningitis remains a common disease worldwide. Approximately 25,000 cases occur annually, of which 70% occur in children under 5 years of age. Despite an apparent recent decline in the incidence of severe neurologic sequelae among children surviving bacterial meningitis, the public health problems as a result of this disease are significant worldwide. The main responsible organisms are *H. influenzae, Streptococcus pneumoniae* and *Neisseria meningitidis*. Community acquired drug resistant *S. pneumoniae* are emerging as a widespread problem in the United States. The peptide alone or in combination with known antibiotics could be administered orally or systemically to treat meningitis. The preferred route would be intravenously either once a day or multiple administration per day. Treatment would preferably last for up to 14 days.

10/ Cystic fibrosis. Cystic fibrosis (CF) is the most common genetic disorder of the Caucasian population. Pulmonary disease is the most common cause of premature death in cystic fibrosis patients. Optimum antimicrobial therapy for CF is not known, and it is generally believed that the introduction of better anti-pseudomonal antibiotics has been the major factor contributing to the increase in life expectancy for CF patients. The most common organisms associated with lung disease in CF are *S. aureus*, *P. aeruginosa* and *H. influenzae*.

The peptide alone or in combination with other antibiotics could be administrated orally or systemically or via aerosol to treat cystic fibrosis. Preferably, treatment is effected for up to 3 weeks during acute pulmonary disease and/or for up to 2 weeks every 2-6 months to prevent acute exacerbations.

11/ Infective endocarditis. Infective endocarditis results from infection of the heart valve cusps, although any part of the endocardium or any prosthetic material inserted into the heart may be involved. It is usually fatal if untreated. Most infections are nosocomial in origin, caused by pathogens increasingly resistant to available drugs. The main organisms responsible are *Viridans streptococci*, *Enterococcus* spp, *S. aureus* and CoNS.

The peptide alone or in combination with other antibiotics could be administered orally or systemically to treat endocarditis, although systemic administration would be preferred. Treatment is preferably for 2-6 weeks in duration and may be given as a continuous infusion or multiple administration during the day.

12/ Osteomyelitis. In early acute disease the vascular supply to the bone is compromised by infection extending into surrounding tissue. Within this necrotic and ischemic tissue, the bacteria may be difficult to eradicate even after an intense host response, surgery, and/or antibiotic therapy. The main organisms responsible are *S. aureus, E. coli*, and *P. aeruginosa*.

The peptide antibiotic could be administered systemically alone or in combination with other antibiotics. Treatment would be 2-6 weeks in duration. The peptide antibiotic could be given as a continuous infusion or multiple administration during the day. Peptide antibiotic could be used as an antibiotic-impregnated cement or as antibiotic coated beads for joint replacement procedures.

13/ Sepsis in immunocompromised host. Treatment of infections in patients who are immunocompromised by virtue of chemotherapy-induced granulocytopenia and immunosuppression related to organ or bone marrow transplantation is always a big challenge. The neutropenic patient is especially susceptible to bacterial infection, so antibiotic therapy should be initiated promptly to cover likely pathogens, if infection is suspected. Organisms likely to cause infections in granulocytopenic patients are: *S. epidermidis, S. aureus, S. viridans, Enterococcus spp, E. coli, Klebsiella spp, P. aeruginosa and Candida spp.*

The peptide alone or with an antibiotic is preferably administered orally or systemically for 2-6 weeks in duration. The peptide antibiotic could be given as a continuous infusion or multiple administration during the day.

Effective treatment of infection may be examined in several different ways. The patient may exhibit reduced fever, reduced number of organisms, lower level of inflammatory molecules (*e.g.*, IFN-γ, IL-12, IL-1, TNF), and the like.

The *in vivo* therapeutic efficacy from administering a cationic peptide and antibiotic agent in combination is based on a successful clinical outcome and does not require 100% elimination of the organisms involved in the infection. Achieving a level of antimicrobial activity at the site of infection that allows the host to survive or eradicate the

microorganism is sufficient. When host defenses are maximally effective, such as in an otherwise healthy individual, only a minimal antimicrobial effect may suffice. Thus, reducing the organism load by even one log (a factor of 10) may permit the defenses of the host to control the infection. In addition, clinical therapeutic success may depend more on augmenting an early bactericidal effect than on the long-term effect. These early events are a significant and critical part of therapeutic success, because they allow time for the host defense mechanisms to activate. This is especially true for life-threatening infections (e.g. meningitis) and other serious chronic infections (e.g. infective endocarditis).

Peptides and antibiotic agents of the present invention are preferably administered as a pharmaceutical composition. Briefly, pharmaceutical compositions of the present invention may comprise one or more of the peptide analogues described herein, in combination with one or more physiologically acceptable carriers, diluents, or excipients. As noted herein, the formulation buffer used may affect the efficacy or activity of the peptide analogue.

The antibiotic agent may be a cytokine, antiviral agent (*e.g.* acyclovir; amantadine hydrochloride; didanosine; edoxudine; famciclovir; foscarnet; ganciclovir; idoxuridine; interferon; lamivudine; nevirapine; penciclovir; podophyllotoxin; ribavirin; rimantadine; sorivudine; stavudine; trifluridine; vidarabine; zalcitabine and zidovudine); an antiparasitic agent (*e.g.*, 8-hydroxyquinoline derivatives; cinchona alkaloids; nitroimidazole derivatives; piperazine derivatives; pyrimidine derivatives and quinoline derivatives); parasitic agent (*e.g.*, albendazole; atovaquone; chloroquine phosphate; diethylcarbamazine citrate; eflornithine; halofantrine; iodoquinol; ivermectin; mebendazole; mefloquine hydrochloride; melarsoprol B; metronidazole; niclosamide; nifurtimox; paromomycin; pentamidine isethionate; piperazine; praziquantel; primaquine phosphate; proguanil; pyrantel pamoate; pyrimethamine; pyrvinium pamoate; quinidine gluconate; quinine sulfate; sodium stibogluconate; suramin and thiabendazole); antifungal agent (*e.g.*, allylamines; imidazoles; pyrimidines and triazoles, 5-fluorocytosine; amphotericin B; butoconazole; chlorphenesin; ciclopirox; clioquinol; clotrimazole; econazole; fluconazole; flucytosine; griseofulvin; itraconazole; ketoconazole; miconazole; naftifine hydrochloride; nystatin; selenium sulfide; sulconazole; terbinafine hydrochloride; terconazole; tioconazole; tolnaftate and undecylenate).

The compositions may be administered in a delivery vehicle. For example, the composition can be encapsulated in a liposome (*see, e.g.*, WO 96/10585; WO 95/35094), complexed with lipids, encapsulated in slow-release or sustained release vehicles, such as poly-galactide, and the like. Within other embodiments, compositions may be prepared as a lyophilizate, utilizing appropriate excipients to provide stability.

Pharmaceutical compositions of the present invention may be administered in various manners. For example, cationic peptides with or without antibiotic agents may be administered by intravenous injection, intraperitoneal injection or implantation, subcutaneous injection or implantation, intradermal injection, lavage, inhalation, implantation, intramuscular injection or implantation, intrathecal injection, bladder wash-out, suppositories, pessaries, topical (*e.g.*, creams, ointments, skin patches, eye drops, ear drops, shampoos) application, enteric, oral, or nasal route. The combination is preferably administered intravenously. Systemic routes include intravenous, intramuscular or subcutaneous injection (including a depot for long-term release), intraocular or retrobulbar, intrathecal, intraperitoneal (*e.g.* by intraperitoneal lavage), transpulmonary using aerosolized or nebulized drug or transdermal. Topical routes include administration in the form of salves, ophthalmic drops, ear drops, or irrigation fluids (for, e.g. irrigation of wounds). The compositions may be applied locally as an injection, drops, spray, tablets, cream, ointment, gel, and the like. They may be administered as a bolus or as multiple doses over a period of time.

The level of peptide in serum and other tissues after administration can be monitored by various well-established techniques such as bacterial, chromatographic or antibody based, such as ELISA, assays.

Pharmaceutical compositions of the present invention are administered in a manner appropriate to the infection or disease to be treated. The amount and frequency of administration will be determined by factors such as the condition of the patient, the cause of the infection, and the severity of the infection. Appropriate dosages may be determined by clinical trials, but will generally range from about 0.1 to 50 mg/kg. The general range of dosages for the antibiotic agents are presented below.

Table 7

ANTIMICROBIAL AGENT	DOSE RANGE
Ciprofloxacin	400-1500mg/day
Gentamicin	3 mg/kg/day
Tobramycin	3 mg/kg/day
Imipenem	1500 mg/kg every 12 h
Piperacillin	24 g/day
Vancomycin, Teicoplanin	6-30 mg/kg/day
Streptomycin	500mg-1g/ every 12 h
Methicillin	100-300 mg/day
Ampicillin, Amoxicillin	250-500 mg/ every 8 h
Penicillin	200,000 units/day
Ceftriaxone	4 g/day
Cefotaxime	12 g/day
Metronidazole	4 g/day
Tetracycline	500 mg/every 6 h
Rifampin	600 mg/day
Fluconazole	150-400 mg/day
Acyclovir	200-400 mg/day
Ribavirin	20 mg/ml (aerosol).
Amantadine-rimantadine	200 mg/day
Metronidazole	2 g/day
Cotrimoxazole	15-20 mg/kg/day
Chloroquine	800 mg/day

In addition, the compositions of the present invention may be used in the manner of common disinfectants or in any situation in which microorganisms are undesirable. For example, these peptides may be used as surface disinfectants, coatings, including covalent bonding, for medical devices, coatings for clothing, such as to inhibit growth of bacteria or repel mosquitoes, in filters for air purification, such as on an airplane, in water purification, constituents of shampoos and soaps, food preservatives, cosmetic preservatives, media preservatives, herbicide or insecticides, constituents of building materials, such as in silicone sealant, and in animal product processing, such as curing of animal hides. As used herein, "medical device" refers to any device for use in a patient, such as an implant or prosthesis. Such devices include, stents, tubing, probes, cannulas, catheters, synthetic vascular grafts, blood monitoring devices, artificial heart valves, needles, and the like.

For these purposes, typically the peptides alone or in conjunction with an antibiotic are included in compositions commonly employed or in a suitable applicator, such as for applying to clothing. They may be incorporated or impregnated into the material during manufacture, such as for an air filter, or otherwise applied to devices. The peptides

and antibiotics need only be suspended in a solution appropriate for the device or article. Polymers are one type of carrier that can be used.

The peptides, especially the labeled analogues, may be used in image analysis and diagnostic assays or for targeting sites in eukaryotic multicellular and single cell cellular organisms and in prokaryotes. As a targeting system, the analogues may be coupled with other peptides, proteins, nucleic acids, antibodies and the like.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

SYNTHESIS PURIFICATION AND CHARACTERIZATION OF CATIONIC PEPTIDES AND ANALOGUES

Peptide synthesis is based on the standard solid-phase Fmoc protection strategy. The instrument employed is a 9050 Plus PepSynthesiser (PerSeptive BioSystems Inc.). Polyethylene glycol polystyrene (PEG-PS) graft resins are employed as the solid phase, derivatized with an Fmoc-protected amino acid linker for C-terminal amide synthesis. HATU (O-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) is used as the coupling reagent. During synthesis, coupling steps are continuously monitored to ensure that each amino acid is incorporated in high yield. The peptide is cleaved from the solid-phase resin using trifluoroacetic acid and appropriate scavengers and the crude peptide is purified using preparative reversed-phase chromatography. Typically the peptide is prepared as the trifluoroacetate salt, but other salts, such as acetate, chloride and sulfate, can also be prepared by salt exchange.

All peptides are analyzed by mass spectrometry to ensure that the product has the expected molecular mass. The product should have a single peak accounting for >95% of the total peak area when subjected to analytical reversed-phase high performance liquid chromatography (RP-HPLC), a separation method that depends on the hydrophobicity of the peptide. In addition, the peptide should show a single band accounting for >90% of the total band intensity when subjected to acid-urea gel electrophoresis, a separation method based on the charge to mass ration of the peptide.

Peptide content, the amount of the product that is peptide rather than retained water, salt or solvent, is measured by quantitative amino acid analysis, free amine derivatization or spectrophotometric quantitation. Amino acid analysis also provides information on the ratio of amino acids present in the peptide, which assists in confirming the authenticity of the peptide.

Peptide analogues and their names are listed below. In this list, and elsewhere, the amino acids are denoted by the one-letter amino acid code and lower case letters represent the D-form of the amino acid.

11A9CN	I L R W P W W P W W P W R R K
11A10CN	W W R W P W W P W R R K
11B19CN	I L R W P W R R W P W R R K
11B20	I L R W P W W P W R R K M I L R W P W W P W R R K A A
11D19CN	C L R W P W W P W R R K
11F5CN	I L R R W V W W V W R R K
11F6CN	I L R W W V W W V W W R R K
11G24CN	L W P W W P W R R K
11G25CN	L R W W W P W R R K
11G26CN	L R W P W W P W
11G27CN	W P W W P W R R K
11G28CN	R W W W P W R R K
11J01CN	R R I W K P K W R L P K R
11J02CN	W R W W K P K W R W P K W

CN suffix = amidated C-terminus

EXAMPLE 2

SYNTHESIS OF MODIFIED PEPTIDES

Cationic peptides, such as indolicidin analogues, are modified to alter the physical properties of the original peptide, either by use of modified amino acids in synthesis or by post-synthetic modification. Such modifications include: acetylation at the N-terminus, Fmoc-derivatized N-terminus, polymethylation, peracetylation, and branched derivatives.

α-N-terminal acetylation. Prior to cleaving the peptide from the resin and deprotecting it, the fully protected peptide is treated with N-acetylimidazole in DMF for 1 hour at room temperature, which results in selective reaction at the α-N-terminus. The peptide is then deprotected/cleaved and purified as for an unmodified peptide.

Fmoc-derivatized α-N-terminus. If the final Fmoc deprotection step is not carried out, the α-N-terminus Fmoc group remains on the peptide. The peptide is then side-chain deprotected/cleaved and purified as for an unmodified peptide.

Polymethylation. The purified peptide in a methanol solution is treated with excess sodium bicarbonate, followed by excess methyl iodide. The reaction mixture is stirred overnight at room temperature, extracted with organic solvent, neutralized and purified as for an unmodified peptide. Using this procedure, a peptide is not fully methylated; methylation of MBI 11CN yielded an average of 6 methyl groups. Thus, the modified peptide is a mixture of methylated products.

Peracetylation. A purified peptide in DMF solution is treated with N-acetylimidazole for 1 hour at room temperature. The crude product is concentrated, dissolved

in water, lyophilized, re-dissolved in water and purified as for an unmodified peptide. Complete acetylation of primary amine groups is observed.

Four/eight branch derivatives. The branched peptides are synthesized on a four or eight branched core bound to the resin. Synthesis and deprotection/cleavage proceed as for an unmodified peptide. These peptides are purified by dialysis against 4 M guanidine hydrochloride then water, and analyzed by mass spectrometry.

EXAMPLE 3

IN VITRO ASSAYS TO MEASURE CATIONIC PEPTIDE ACTIVITY

A cationic peptide may be tested for antimicrobial activity alone before assessing its enhancing activity with antibiotic agents. Preferably, the peptide has measurable antimicrobial activity.

Agarose Dilution Assay

The agarose dilution assay measures antimicrobial activity of peptides and peptide analogues, which is expressed as the minimum inhibitory concentration (MIC) of the peptides.

In order to mimic *in vivo* conditions, calcium and magnesium supplemented Mueller Hinton broth is used in combination with a low EEO agarose as the bacterial growth medium. Agarose, rather than agar, is used as the charged groups in agar prevent peptide diffusion through the media. The media is autoclaved and then cooled to 50 - 55° C in a water bath before aseptic addition of antimicrobial solutions. The same volume of different concentrations of peptide solution are added to the cooled molten agarose that is then poured to a depth of 3 - 4 mm.

The bacterial inoculum is adjusted to a 0.5 McFarland turbidity standard (PML Microbiological) and then diluted 1:10 before application on to the agarose plate. The final inoculum applied to the agarose is approximately 10^4 CFU in a 5 - 8 mm diameter spot. The agarose plates are incubated at 35 - 37°C for 16 to 20 hours.

The MIC is recorded as the lowest concentration of peptide that completely inhibits growth of the organism as determined by visual inspection. Representative MICs for various indolicidin analogues against bacteria are shown in Table 8 and representative MICs against Candida are shown in Table 9 below.

Table 8

1. MBI 11A9CN

Organism	Organism #	MIC (µg/ml)
A. calcoaceticus	AC002	8
E. cloacae	ECL007	128
E. coli	ECO005	32
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	32
P. aeruginosa	PA004	128
S. aureus	SA014	4
S. aureus	SA093	2
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

2. MBI 11A10CN

Organism	Organism #	MIC (µg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	64
E. coli	ECO005	16
E. faecalis	EFS001	4
E. faecalis	EFS008	16
K. pneumoniae	KP001	16
P. aeruginosa	PA004	64
S. aureus	SA014	4
S. aureus	SA093	2
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

3. MBI 11B7ACN

Organism	Organism #	MIC (µg/ml)
A. calcoaceticus	AC002	2
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	1
E. faecalis	EFS008	8
K. pneumoniae	KP001	8
P. aeruginosa	PA004	128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

4. MBI 11B7CNF12

Organism	Organism #	MIC (µg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	16
P. aeruginosa	PA004	>128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

5. MBI 11B19CN

Organism	Organism #	MIC (µg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	32
K. pneumoniae	KP001	64
P. aeruginosa	PA004	128
S. aureus	SA014	4
S. aureus	SA093	2
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

6. MBI 11B20

Organism	Organism #	MIC (µg/ml)
A. calcoaceticus	AC002	32
E. cloacae	ECL007	128
E. coli	ECO005	32
E. faecalis	EFS001	8
E. faecalis	EFS008	32
K. pneumoniae	KP001	64
P. aeruginosa	PA004	128
S. aureus	SA014	32
S. aureus	SA093	4
S. epidermidis	SE010	32
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

7. MBI 11D19CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	8
E. cloacae	ECL007	>128
E. coli	ECO005	32
E. faecalis	EFS001	4
E. faecalis	EFS008	64
K. pneumoniae	KP001	32
P. aeruginosa	PA004	128
S. aureus	SA014	4
S. aureus	SA093	2
S. epidermidis	SE010	8
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

8. MBI 11F4

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	8
P. aeruginosa	PA004	128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	16
S. marcescens	SMS003	>128

9. MBI 11F5CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	8
P. aeruginosa	PA004	32
S. aureus	SA014	4
S. aureus	SA093	2
S. epidermidis	SE010	4
S. maltophilia	SMA002	16
S. marcescens	SMS003	>128

10. MBI 11F6CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	16
E. cloacae	ECL007	64
E. coli	ECO005	32
E. faecalis	EFS001	16
E. faecalis	EFS008	16
K. pneumoniae	KP001	32
P. aeruginosa	PA004	128
S. aureus	SA014	16
S. aureus	SA093	8
S. epidermidis	SE010	8
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

11. MBI 11G24CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	16
P. aeruginosa	PA004	128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

12. MBI 11G25CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	2
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	16
K. pneumoniae	KP001	16
P. aeruginosa	PA004	64
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	2
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

13. MBI 11G26CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	2
E. cloacae	ECL007	>128
E. coli	ECO005	32
E. faecalis	EFS001	2
E. faecalis	EFS008	4
K. pneumoniae	KP001	32
P. aeruginosa	PA004	>128
S. aureus	SA014	4
S. aureus	SA093	0.5
S. epidermidis	SE010	4
S. maltophilia	SMA002	128
S. marcescens	SMS003	>128

14. MBI 11G27CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	2
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	8
E. faecalis	EFS008	32
K. pneumoniae	KP001	32
P. aeruginosa	PA004	128
S. aureus	SA014	4
S. aureus	SA093	1
S. epidermidis	SE010	8
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

15. MBI 11G28CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	2
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	4
E. faecalis	EFS008	32
K. pneumoniae	KP001	64
P. aeruginosa	PA004	128
S. aureus	SA014	4
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

16. MBI 11H01CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	2
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	8
P. aeruginosa	PA004	128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	2
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

17. MBI 11H02CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	16
K. pneumoniae	KP001	32
P. aeruginosa	PA004	>128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

18. MBI 11H03CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	8
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	16
P. aeruginosa	PA004	>128
S. aureus	SA014	2
S. aureus	SA093	2
S. epidermidis	SE010	4
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

19. MBI 11H04CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	8
E. cloacae	ECL007	>128
E. coli	ECO005	32
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	64
P. aeruginosa	PA004	>128
S. aureus	SA014	4
S. aureus	SA093	2
S. epidermidis	SE010	16
S. maltophilia	SMA002	>128
S. marcescens	SMS003	>128

20. MBI 11H05CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	8
P. aeruginosa	PA004	128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	16
S. marcescens	SMS003	>128

21. MBI 11H06CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	8
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	16
K. pneumoniae	KP001	64
P. aeruginosa	PA004	>128
S. aureus	SA014	8
S. aureus	SA093	1
S. epidermidis	SE010	8
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

22. MBI 11H07CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	8
E. cloacae	ECL007	>128
E. coli	ECO005	32
E. faecalis	EFS001	4
E. faecalis	EFS008	16
K. pneumoniae	KP001	128
P. aeruginosa	PA004	>128
S. aureus	SA014	8
S. aureus	SA093	2
S. epidermidis	SE010	16
S. maltophilia	SMA002	128
S. marcescens	SMS003	>128

23. MBI 11H08CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	8
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	32
P. aeruginosa	PA004	>128
S. aureus	SA014	4
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	32
S. marcescens	SMS003	>128

24. MBI 11H09CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	32
E. faecalis	EFS001	4
E. faecalis	EFS008	64
K. pneumoniae	KP001	64
P. aeruginosa	PA004	>128
S. aureus	SA014	8
S. aureus	SA093	2
S. epidermidis	SE010	16
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

25. MBI 11H10CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	16
P. aeruginosa	PA004	>128
S. aureus	SA014	4
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

26. MBI 11H11CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	16
P. aeruginosa	PA004	>128
S. aureus	SA014	4
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

27. MBI 11H12CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	16
E. faecalis	EFS001	2
E. faecalis	EFS008	8
K. pneumoniae	KP001	16
P. aeruginosa	PA004	>128
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	4
S. maltophilia	SMA002	64
S. marcescens	SMS003	>128

28. MBI 11J01CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	>128
E. coli	ECO005	64
E. faecalis	EFS001	128
E. faecalis	EFS008	>128
K. pneumoniae	KP001	>128
P. aeruginosa	PA004	>128
S. aureus	SA014	16
S. aureus	SA093	2
S. epidermidis	SE010	32
S. maltophilia	SMA002	>128
S. marcescens	SMS003	>128

29. MBI 11J02CN

Organism	Organism #	MIC (μg/ml)
A. calcoaceticus	AC002	4
E. cloacae	ECL007	64
E. coli	ECO005	4
E. faecalis	EFS001	4
E. faecalis	EFS008	16
K. pneumoniae	KP001	4
P. aeruginosa	PA004	32
S. aureus	SA014	2
S. aureus	SA093	1
S. epidermidis	SE010	2
S. maltophilia	SMA002	8
S. marcescens	SMS003	>128

Table 9

Organism	MBI 11CN MIC (μg/ml)	MBI 11B7CN MIC (μg/ml)
C. albicans CA001	128	64
C. albicans CA002	64	32
C. albicans CA003	128	64
C. albicans CA004	64	32
C. albicans CA005	128	32
C. albicans CA006	128	64
C. albicans CA007	128	64
C. albicans CA008	64	32
C. albicans CA009	64	32
C. albicans CA010	128	64
C. albicans CA011	64	64
C. albicans CA012	128	64
C. albicans CA013	128	64
C. albicans CA014	64	32
C. albicans CA015	128	64
C. albicans CA016	128	64
C. albicans CA017	128	64
C. albicans CA018	128	64
C. albicans CA019	128	64
C. albicans CA020	128	32
C. albicans CA021	128	32
C. albicans CA022	32	32
C. albicans CA023	128	64
C. albicans CA024	16	8
C. glabrata CGL001	>128	128
C. glabrata CGL002	>128	128
C. glabrata CGL003	>128	128
C. glabrata CGL004	>128	128
C. glabrata CGL005	>128	128
C. glabrata CGL009	>128	128
C. glabrata CGL010	>128	128
C. krusei CKR001	0.5	1
C. tropicalis CTR001	4	4
C. tropicalis CTR002	4	8
C. tropicalis CTR003	8	8
C. tropicalis CTR004	4	8
C. tropicalis CTR005	4	4
C. tropicalis CTR006	16	8
C. tropicalis CTR007	16	8
C. tropicalis CTR008	8	4
C. tropicalis CTR009	8	4

Broth Dilution Assay

Typically 100 μl of calcium and magnesium supplemented Mueller Hinton broth is dispensed into each well of a 96-well microtitre plate and 100 μl volumes of two-fold serial dilutions of the peptide are prepared across the plate. One row of wells receives no peptide and is used as a growth control. Each well is inoculated with approximately 5×10^5 CFU of bacteria and the plate is incubated at 35 - 37°C for 16-20 hours. The MIC is recorded at the lowest concentration of peptide that completely inhibits growth of the organism as determined by visual inspection.

Time Kill Assay

Time kill curves are used to determine the antimicrobial activity of cationic peptides over a time interval. Briefly, in this assay, a suspension of microorganisms equivalent to a 0.5 McFarland Standard is prepared in 0.9% saline. This suspension is then diluted such that when added to a total volume of 9 ml of cation-adjusted Mueller Hinton broth, the inoculum size is 1×10^6 CFU/ml. An aliquot of 0.1 ml is removed from each tube at pre-determined intervals up to 24 hours, diluted in 0.9% saline and plated in triplicate to determine viable colony counts. The number of bacteria remaining in each sample is plotted over time to determine the rate of cationic peptide killing. Generally a three or more $\log_{10}$ reduction in bacterial counts in the antimicrobial suspension compared to the growth controls indicate an adequate bactericidal response.

As shown in Figures 1A-D, most of the peptides demonstrate a three or more $\log_{10}$ reduction in bacterial counts in the antimicrobial suspension compared to the growth controls, indicating that these peptides have met the criteria for a bactericidal response.

EXAMPLE 4

ASSAYS TO MEASURE ENHANCED ACTIVITY OF ANTIBIOTIC AGENT AND CATIONIC PEPTIDE COMBINATIONS

Killing Curves

Time kill curves resulting from combination of cationic peptide and antibiotic agent are compared to that resulting from agent alone.

The assay is performed as described above except that duplicate tubes are set up for each concentration of the antibiotic agent alone and of the combination of antibiotic

agent and cationic peptide. Synergy is demonstrated by at least a 100-fold (2 $\log_{10}$) increase in killing at 24 hours by the antibiotic agent and cationic peptide combination compared to the antibiotic agent alone. A time kill assay is shown in Figure 1E for MBI 26 in combination with vancomycin against a bacterial strain. The combination of peptide and antibiotic agent gave greater killing than either peptide or antibiotic agent alone.

FIC Measurements

In this method, synergy is determined using the agarose dilution technique. An array of plates or tubes, each containing a combination of peptide and antibiotic in a unique concentration mix, is inoculated with bacterial isolates. When performing solid phase assays, calcium and magnesium supplemented Mueller Hinton broth is used in combination with a low EEO agarose as the bacterial growth medium. Broth dilution assays can also be used to determine synergy. Synergy is determined for cationic peptides in combination with a number of conventional antibiotic agents, for example, penicillins, cephalosporins, carbapenems, monobactams, aminoglycosides, macrolides, fluoroquinolones, nisin and lysozyme.

Synergy is expressed as a fractional inhibitory concentration (FIC), which is calculated according to the equation below. An FIC ≤ 0.5 is evidence of synergy. An additive response has an FIC value > 0.5 and ≤ 1, while an indifferent response has an FIC value >1 and ≤ 2.

$$FIC = \frac{MIC\ (peptide\ in\ combination)}{MIC\ (peptide\ alone)} + \frac{MIC\ (antibiotic\ in\ combination)}{MIC\ (antibiotic\ alone)}$$

Tables 10, 11 and 12 present combinations of cationic peptides and antibiotic agents that display an FIC value of less than or equal to 1. Although FIC is measured *in vitro* and synergy defined as an FIC of less than or equal to 0.5, an additive effect may be therapeutically useful. As shown below, although all the microorganisms are susceptible (NCCLS breakpoint definitions) to the tested antibiotic agents, the addition of the cationic peptide improves the efficacy of the antibiotic agent.

Table 10

Microorganism	Strain	Antibiotic	FIC	Peptide
S. aureus	SA014	Ciprofloxacin	0.63	MBI 26
S. aureus	SA014	Ciprofloxacin	0.75	MBI 28
S. aureus	SA014	Ciprofloxacin	1.00	MBI 11A2CN

Microorganism	Strain	Antibiotic	FIC	Peptide
S. aureus	SA093	Ciprofloxacin	0.75	MBI 11A2CN
S. aureus	SA7609	Clindamycin	0.25	MBI 26
S. aureus	SA7609	Methicillin	0.56	MBI 26
S. aureus	SA7610	Clindamycin	0.63	MBI 26
S. aureus	SA7610	Methicillin	0.31	MBI 26
S. aureus	SA7795	Ampicillin	0.52	MBI 26
S. aureus	SA7795	Clindamycin	0.53	MBI 26
S. aureus	SA7796	Ampicillin	1.00	MBI 26
S. aureus	SA7796	Clindamycin	0.51	MBI 26
S. aureus	SA7817	Ampicillin	0.50	MBI 26
S. aureus	SA7818	Ampicillin	1.00	MBI 26
S. aureus	SA7818	Erythromycin	0.15	MBI 26
S. aureus	SA7818	Erythromycin	0.15	MBI 26
S. aureus	SA7821	Erythromycin	0.50	MBI 26
S. aureus	SA7821	Erythromycin	0.50	MBI 26
S. aureus	SA7822	Ampicillin	0.25	MBI 26
S. aureus	SA7823	Ampicillin	0.25	MBI 26
S. aureus	SA7824	Ampicillin	1.00	MBI 26
S. aureus	SA7825	Ampicillin	1.00	MBI 26
S. aureus	SA7825	Erythromycin	1.00	MBI 26
S. aureus	SA7825	Erythromycin	1.00	MBI 26
S. aureus	SA7834	Ampicillin	0.53	MBI 26
S. aureus	SA7834	Clindamycin	0.56	MBI 26
S. aureus	SA7835	Ampicillin	0.53	MBI 26
S. aureus	SA7836	Ampicillin	0.75	MBI 26
S. aureus	SA7837	Ampicillin	1.00	MBI 26
S. aureus	SAATCC25293	Methicillin	0.50	MBI 26
S. aureus	SAATCC29213	Methicillin	0.31	MBI 26
S. aureus	SAW1133	Methicillin	0.75	MBI 26
S. epidermidis	SE8406	Clindamycin	0.50	MBI 26
S. epidermidis	SE8416	Ampicillin	0.52	MBI 31
S. epidermidis	SE8416	Clindamycin	0.56	MBI 26
S. epidermidis	SE8505	Ampicillin	1.00	MBI 26
S. epidermidis	SE8565	Ampicillin	1.00	MBI 26
S. epidermidis	SH8575	Ampicillin	0.27	MBI 31
S. haemolyticus	SA7797	Ampicillin	0.50	MBI 31
S. haemolyticus	SA7817	Ampicillin	0.26	MBI 31
S. haemolyticus	SA7818	Ampicillin	0.52	MBI 31
S. haemolyticus	SA7834	Ampicillin	0.52	MBI 31
S. haemolyticus	SA7835	Ampicillin	0.50	MBI 31
S. haemolyticus	SH8459	Ampicillin	0.52	MBI 26
S. haemolyticus	SH8472	Ampicillin	0.56	MBI 26
S. haemolyticus	SH8563	Ampicillin	0.75	MBI 26

Microorganism	Strain	Antibiotic	FIC	Peptide
S. haemolyticus	SH8564	Ampicillin	0.62	MBI 26
S. haemolyticus	SH8575	Ampicillin	0.75	MBI 26
S. haemolyticus	SH8576	Ampicillin	0.62	MBI 26
S. haemolyticus	SH8578	Ampicillin	1.00	MBI 26
S. haemolyticus	SH8597	Ampicillin	1.00	MBI 31

Table 11

Microorganism	Strain	Teicoplanin (μg/ml)		MBI 26 (μg/ml)	
		Alone	+ MBI 26	Alone	+ Teicoplanin
E. faecium 97001	VanB	0.5	0.25	64	4
E. faecium 97002	VanB	0.5	0.25	64	1
E. faecium 97003	VanB	0.5	0.25	64	1
E. faecium 97005	VanB	1	0.25	64	2
E. faecium 97006	VanB	0.5	0.5	64	4
E. faecium 97007	VanB	0.5	0.25	64	1
E. faecium 97008	VanB	0.5	0.25	64	4
E. faecium 97009	VanB	0.5	0.25	32	1
E. faecium 97010	VanB	0.5	0.25	64	4
E. faecium 97011	VanB	0.5	0.25	64	4
E. faecium 97012	VanB	8	0.25	64	4
E. faecium 97013	VanB	8	0.25	64	8
E. faecium 97014	VanB	8	0.25	32	4
E. faecium 97015	VanB	0.5	0.25	64	4
E. faecium 97016	VanB	0.5	0.25	64	4
E. faecalis 97040	VanB	0.5	0.25	64	8
E. faecalis 97041	VanB	1	0.25	64	8
E. faecalis 97042	VanB	1	0.25	64	8
E. faecalis 97043	VanB	0.5	0.25	64	8

Table 12

1. Amikacin

Peptide	Organism	FIC	Amikacin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Amikacin
MBI 11B16CN	*A. baumannii* ABI001	0.50	32	0.125	32	16
	A. baumannii ABI003	0.53	16	0.5	16	8
	P. aeruginosa PA022	0.38	64	8	64	16
	P. aeruginosa PA037	0.25	16	2	>128	32
	S. maltophilia SMA018	0.31	128	8	32	8
	S. maltophilia SMA022	0.09	>128	8	>128	16
	E. faecalis EFS008	0.28	32	8	8	0.25
MBI 21A2	*A. baumannii* ABI001	0.52	64	32	8	0.125
	A. baumannii ABI003	0.52	16	8	8	4
	P. aeruginosa PA022	0.50	64	16	8	2
	S. maltophilia SMA018	0.50	>128	64	16	4
	S. maltophilia SMA022	0.25	>128	32	>128	32
	E. faecium EFM004	0.56	128	64	>128	16
	E. faecalis EFS008	0.50	64	32	>128	0.125
	S. aureus SA025 MRSA	0.56	32	2	2	1
	S. epidermidis SE003	0.38	32	4	>128	64
MBI 26	*A. baumannii* ABI001	0.50	32	8	8	2
	A. baumannii ABI003	0.38	16	2	8	2
	S. maltophilia SMA021	0.13	128	8	32	2
	S. maltophilia SMA037	0.19	128	16	>128	16
MBI 27	*A. baumannii* ABI003	0.52	16	0.25	8	4
	B. cepacia BC005	0.50	64	16	>128	64
	S. maltophilia SMA037	0.31	64	4	64	16
	S. maltophilia SMA060	0.50	>128	0.125	16	8
	E. faecalis EFS008	0.53	32	1	4	2
MBI 29A3	*B. cepacia* BC003	0.50	32	8	>128	64
	B. cepacia BC005	0.38	128	32	>128	32
	S. maltophilia SMA036	0.38	>128	32	64	16
	S. maltophilia SMA063	0.56	>128	16	8	4
	S. maltophilia SMA064	0.56	>128	16	8	4
	E. faecium EFM004	0.56	128	8	8	4
MBI 29F1	*A. baumannii* ABI001	0.51	32	0.25	8	4
	A. baumannii ABI003	0.63	16	2	4	2
	E. coli ECO022	0.51	16	0.125	4	2
	P. aeruginosa PA022	0.53	128	64	4	0.125
	S. maltophilia SMA021	0.31	128	8	8	2
	S. maltophilia SMA022	0.31	>128	16	16	4
	E. faecium EFM004	0.38	>128	32	32	8
	E. faecalis EFS008	0.28	64	16	4	0.125
	S. aureus SA014 MRSA	0.53	32	16	4	0.125
	S. epidermidis SE002	0.38	64	16	32	4
	S. epidermidis SE003	0.50	64	16	32	8

2. Ceftriaxone

Peptide	Organism	FIC	Ceftriaxone MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Ceftriaxone
MBI 11B7CN	*A. baumannii* ABI002	0.50	32	8	32	8
	A. baumannii ABI006	0.25	128	16	32	4
	B. cepacia BC003	0.52	32	16	>128	4
	P. aeruginosa PA008	0.25	128	16	128	16
	P. aeruginosa PA024	0.50	64	32	>128	0.125
	S. maltophilia SMA020	0.75	>128	64	16	8
	S. maltophilia SMA021	0.50	>128	64	32	8
	S. maltophilia SMA023	0.38	128	32	128	16
MBI 11J02CN	*A. baumannii* ABI005	0.56	16	8	8	0.5
	B. cepacia BC003	0.50	16	4	>128	64
	E. cloacae ECL014	0.38	128	16	32	8
	E. cloacae ECL015	0.50	64	16	32	8
	P. aeruginosa PA008	0.50	64	0.125	64	32
	P. aeruginosa PA039	0.50	64	16	64	16
	S. aureus SA025 MRSA	0.52	8	0.125	2	1
	S. epidermidis SE012	0.50	64	16	4	1
	S. epidermidis SE073	0.38	128	16	4	1
MBI 26	*A. baumannii* ABI002	0.50	64	16	8	2
	A. baumannii ABI005	0.56	16	8	2	0.125
	B. cepacia BC003	0.50	16	8	>128	0.125
	E. cloacae ECL014	0.50	128	32	8	2
	E. cloacae ECL015	0.19	64	4	32	4
	K. pneumoniae KP003	0.56	8	4	16	1
	P. aeruginosa PA008	0.13	64	8	128	0.125
	P. aeruginosa PA024	0.50	16	4	128	32
	S. maltophilia SMA019	0.50	>128	64	4	1
	S. maltophilia SMA020	0.38	>128	32	4	1
	S. aureus SA025 MRSA	0.52	8	0.125	1	0.5
	S. epidermidis SE007	0.27	8	2	32	0.5
	S. epidermidis SE012	0.27	64	16	64	1

3. Ciprofloxacin

Peptide	Organism	FIC	Ciprofloxacin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Ciprofloxacin
MBI 11A1CN	*S. aureus* SA10	0.53	32	16	128	4
	S. aureus SA11	0.50	64	32	>128	1
MBI 11D18CN	*P. aeruginosa* PA24	0.31	16	4	>128	16
	P. aeruginosa PA77	0.50	2	0.5	128	32
MBI 21A1	*S. aureus* SA25	0.16	4	0.125	32	4
	S. aureus SA93	0.50	32	8	4	1
	P. aeruginosa PA4	0.50	0.5	0.125	128	32
	P. aeruginosa PA41	0.50	4	1	16	4

MBI 21A2	*S. aureus* SA25	0.50	2	0.5	16	4
	S. aureus SA93	0.38	32	8	16	2
	P. aeruginosa PA4	0.50	0.5	0.125	>128	64
	P. aeruginosa PA41	0.50	4	1	64	16
MBI 26	*S. aureus* SA11	0.50	64	32	128	0.125
	P. aeruginosa PA41	0.50	4	1	128	32
	P. aeruginosa PA77	0.56	2	0.125	128	64
	A. calcoaceticus 1	0.51	0.5	0.25	>64	1
	A. calcoaceticus 6	0.50	1	0.25	>32	16
	E. cloacae 13	0.27	1	0.25	>128	4
	E. cloacae 15	0.38	1	0.25	>32	8
	E. cloacae 16	0.38	2	0.25	>32	16
	P. aeruginosa 23	0.53	1	0.5	>32	2
	P. aeruginosa 24	0.53	1	0.5	>32	2
	S. maltophilia 34	0.25	2	0.25	>32	8
	S. maltophilia 35	0.50	2	0.5	>32	16
MBI 27	*S. aureus* SA10	0.75	32	8	2	1
	S. aureus SA93	0.63	32	4	2	1
	P. aeruginosa PA4	0.75	0.5	0.25	32	8
MBI 28	*S. aureus* SA11	0.63	32	16	64	8
	S. aureus SA25	0.56	2	0.125	2	1
	P. aeruginosa PA24	0.75	32	8	64	32
MBI 29	*S. aureus* SA10	0.38	32	4	4	1
	S. aureus SA93	0.50	32	8	2	0.5
	P. aeruginosa PA41	0.52	8	4	8	0.125
	P. aeruginosa PA77	0.50	2	0.5	64	16
	A. calcoaceticus 5	0.56	2	1	16	1
	A. calcoaceticus 9	0.56	2	1	16	1
	E. cloacae 14	0.50	1	0.25	>16	8
	E. cloacae 15	0.50	1	0.25	>16	8
	P. aeruginosa 30	0.56	4	0.25	>16	16
	P. aeruginosa 31	0.53	16	0.5	>16	16
	S. maltophilia 34	0.27	2	0.5	>16	0.5
	S. maltophilia 35	0.63	2	0.25	>16	16
	S. maltophilia 36	0.56	8	0.5	>16	16
MBI 29A2	*S. aureus* SA10	0.52	32	0.5	4	2
	S. aureus SA93	0.50	32	8	2	0.5
	P. aeruginosa PA24	0.63	32	16	64	8
MBI 29A3	*S. aureus* SA10	0.75	32	16	2	0.5
	S. aureus SA25	0.63	4	2	1	0.125
	P. aeruginosa PA24	0.50	32	16	64	0.125
	P. aeruginosa PA41	0.63	4	0.5	8	4

4. Gentamicin

Peptide	Organism	FIC	Gentamicin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Gentamicin
MBI 11A1CN	*S. maltophilia* SMA019	0.31	8	2	>128	16
	S. maltophilia SMA020	0.31	8	2	>128	16
	E. faecium EFM004	0.28	>128	64	32	1
	S. aureus SA014 MRSA	0.56	32	2	8	4
	S. epidermidis SE074	0.51	128	1	32	16
MBI 11B16CN	*A. baumannii* ABI001	0.31	64	4	16	4
	A. baumannii ABI002	0.31	32	2	16	4
	A. calcoaceticus AC001	0.25	8	1	32	4
	P. aeruginosa PA022	0.38	32	8	64	8
	P. aeruginosa PA041	0.31	8	2	>128	16
	S. maltophilia SMA016	0.31	>128	64	>128	16
	S. maltophilia SMA019	0.38	64	8	32	8
	E. faecalis EFS008	0.38	>128	64	4	0.5
	S. aureus SA014 MRSA	0.53	32	1	8	4
MBI 11D18CN	*A. baumannii* ABI001	0.27	64	16	32	0.5
	A. baumannii ABI002	0.56	16	8	32	2
	E. coli ECO006	0.27	64	16	8	0.125
	K. pneumonia KP020	0.50	64	32	32	0.125
	P. aeruginosa PA022	0.52	16	8	8	0.125
	P. aeruginosa PA041	0.14	8	0.125	64	8
	S. maltophilia SMA016	0.38	128	16	64	16
	S. maltophilia SMA019	0.19	32	4	8	0.5
	E. faecium EFM004	0.05	>128	8	8	0.125
	E. faecalis EFS008	0.19	128	8	2	0.25
	S. aureus SA014 MRSA	0.13	32	2	2	0.125
	S. aureus SA025 MRSA	0.14	64	1	1	0.125
	S. epidermidis SE071	0.27	16	4	8	0.125
	S. epidermidis SE074	0.09	64	4	4	0.125
MBI 21A2	*A. baumannii* ABI002	0.56	32	16	8	0.5
	P. aeruginosa PA022	0.50	32	8	8	2
	S. maltophilia SMA019	0.50	64	16	16	4
	S. maltophilia SMA020	0.50	64	16	16	4
	S. maltophilia SMA021	0.50	64	16	16	4
	S. aureus SA025 MRSA	0.63	64	32	8	1
MBI 26	*A. baumannii* ABI001	0.50	64	16	8	2
	A. baumannii ABI002	0.53	16	0.5	8	4
	P. aeruginosa PA041	0.63	8	1	64	32
	S. maltophilia SMA016	0.25	>128	32	>128	32
	S. maltophilia SMA017	0.38	64	16	16	2

MBI 27	*A. baumannii* ABI002	0.52	32	0.5	8	4
	P. aeruginosa PA022	0.52	32	16	8	0.125
	S. maltophilia SMA016	0.50	>128	64	64	16
	S. maltophilia SMA017	0.52	128	64	8	0.125
	E. faecalis EFS008	0.38	>128	64	4	0.5
	S. aureus SA014 MRSA	0.50	32	0.125	2	1
MBI 29	*S. maltophilia* SMA019	0.53	32	16	4	0.125
	S. maltophilia SMA020	0.53	32	16	4	0.125
	E. faecalis EFS008	0.38	128	32	1	0.125
	S. epidermidis SE074	0.50	128	0.5	4	2
MBI 29A3	*S. maltophilia* SMA019	0.31	64	16	2	0.125
	S. maltophilia SMA021	0.31	64	16	2	0.125
MBI 29F1	*P. aeruginosa* PA023	0.52	8	0.125	128	64
	S. maltophilia SMA016	0.56	>128	16	32	16
	S. maltophilia SMA017	0.53	64	32	4	0.125
Deber A2KA2	*A. baumannii* ABI001	0.53	64	32	>128	8
	A. baumannii ABI002	0.50	64	32	>128	0.125
	A. calcoaceticus AC001	0.56	8	4	>128	16
	P. aeruginosa PA022	0.52	32	16	>128	4
	P. aeruginosa PA041	0.50	16	8	>128	0.125
	S. maltophilia SMA017	0.50	128	64	>128	0.125
	S. maltophilia SMA020	0.50	128	64	>128	0.125

5. Mupirocin

Peptide	Organism	FIC	Mupirocin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Mupirocin
MBI 11A1CN	*E. coli* SBECO2	0.05	>100	30	128	2
	E. coli ECO1	0.14	>100	10	32	4
MBI 11A3CN	*E. coli* SBECO1	0.43	100	30	64	8
MBI 11B4CN	*E. coli* SBECO1	0.36	100	30	8	0.5
	E. coli SBECO2	0.09	>100	30	32	2
MBI 11D18CN	*E. coli* SBECO1	0.36	100	30	2	0.125
	E. coli SBECO2	0.06	>100	30	16	0.5
	P. aeruginosa SBPA1	0.35	>100	100	128	32
	P. aeruginosa PA4	0.53	>100	30	128	64
	S. marcescens SBSM1	0.16	>100	100	>128	16
	S. marcescens SBSM2	0.35	>100	100	>128	64
MBI 11G13CN	*E. coli* SBECO2	0.16	>100	30	64	8
	E. coli ECO5	0.43	100	30	64	8
MBI 21A1	*E. coli* SBECO2	0.28	>100	30	8	2
	E. coli ECO3	0.28	100	3	8	2
	P. aeruginosa SBPA1	0.53	>100	30	64	32

MBI 26	*E. coli* SBECO2	0.16	>100	30	8	1
	E. coli ECO5	0.43	100	30	8	1
	P. aeruginosa PA2	0.51	>100	10	128	64
	P. aeruginosa PA4	0.23	>100	100	>128	32
	S. aureus SBSA4	0.28	>100	30	32	8
MBI 27	*E. coli* SBECO2	0.51	>100	10	4	2
	P. aeruginosa PA2	0.25	>100	0.1	64	16
	P. aeruginosa PA4	0.50	>100	0.3	32	16
	S. aureus SBSA3	0.23	100	10	16	2
	S. aureus SBSA4	0.50	>100	0.3	4	2
MBI 28	*E. coli* SBECO1	0.50	100	0.1	4	2
	E. coli ECO2	0.33	100	30	4	0.125
	P. aeruginosa SBPA1	0.53	>100	30	32	16
	P. aeruginosa PA4	0.50	>100	3	32	16
	S. aureus SBSA4	0.51	>100	10	4	2
MBI 29	*S. marcescens* SBSM1	0.23	>100	100	>128	32
	S. aureus SBSA3	0.35	100	10	16	4
	S. aureus SBSA4	0.51	>100	10	4	2
MBI 29A3	*P. aeruginosa* PA2	0.50	>100	0.1	32	16
	P. aeruginosa PA3	0.50	>100	0.1	16	8
	S. marcescens SBSM1	0.16	>100	100	>128	16
	S. marcescens SBSM2	0.35	>100	100	>128	64

6. Piperacillin

Peptide	Organism	FIC	Piperacillin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Piperacillin
MBI 11B7CN	*E. cloacae* 6	0.56	>128	16	32	16
	E. cloacae 9	0.50	>128	1	32	16
	E. cloacae 10	0.50	>128	0.5	32	16
	S. maltophilia 5	0.50	>128	64	>128	64
	S. maltophilia 9	0.50	>128	64	>128	64
	S. maltophilia 11	0.38	>128	64	>128	32
	S. marcescens 1	0.27	32	8	>128	4
	P. aeruginosa 23	0.56	32	2	128	64
	H. influenzae 1	0.50	64	32	>128	0.125
	H. influenzae SB1	0.50	0.5	0.25	>128	0.125
	S. aureus 19 MRSA	0.50	128	32	4	1

MBI 11B9CN	*A. calcoaceticus* 3	0.56	64	32	32	2
	S. maltophilia 5	0.50	>128	64	>128	64
	S. maltophilia 13	0.38	>128	64	>128	32
	S. marcescens SB1	0.26	64	16	>128	2
	P. aeruginosa 15	0.50	>128	64	>128	64
	P. aeruginosa 23	0.13	128	16	64	0.5
	H. influenzae 3	0.50	0.5	0.25	>128	0.125
	H. influenzae SB1	0.50	0.5	0.25	>128	0.125
	*S. aureus*19 MRSA	0.38	128	16	4	1
	S. aureus SB2MRSA	0.56	128	8	2	1
MBI 11CN	*P. aeruginosa* 22	0.52	>128	4	64	32
	P. aeruginosa 23	0.53	128	64	128	4
	S. aureus 18 MRSA	0.50	>128	0.5	32	16
	S. aureus 19 MRSA	0.38	>128	64	8	1
MBI 11D18CN	*A. calcoaceticus 3*	0.38	64	8	32	8
	E. cloacae 9	0.31	>128	16	64	16
	E. cloacae 10	0.50	>128	64	32	8
	S. maltophilia 2	0.50	64	16	32	8
	S. marcescens 1	0.14	64	8	>128	4
	P. aeruginosa 23	0.38	128	32	64	8
	P. aeruginosa 41	0.56	64	32	>128	16
	H. influenzae 3	0.53	0.5	0.25	>128	8
	H. influenzae SB1	0.52	0.5	0.25	>128	4
	*S. aureus*19 MRSA	0.38	128	16	4	1
	S. aureus SB2MRSA	0.50	128	32	2	0.5
MBI 11E3CN	*S. maltophilia* 11	0.51	>128	2	128	64
	S. marcescens SB1	0.26	64	16	>128	2
	P. aeruginosa 23	0.27	128	32	64	1
	P. aeruginosa 32	0.63	64	32	64	8
	H. influenzae 1	0.52	64	32	>128	4
	H. influenzae 2	0.31	32	8	>128	16
	S. aureus 19 MRSA	0.50	>128	64	4	1
MBI 11F3CN	*P. aeruginosa* 23	0.51	128	64	64	0.5
	P. aeruginosa 41	0.63	32	4	128	64
	*S. aureus*19 MRSA	0.38	>128	32	4	1
	S. aureus SB3MRSA	0.50	>128	64	8	2
MBI 11F4CN	*E. cloacae* 10	0.52	>128	4	16	8
	S. maltophilia 2	0.53	64	32	16	0.5
	S. marcescens 1	0.25	>128	64	>128	0.5
	P. aeruginosa 7	0.38	>128	64	64	8
	P. aeruginosa 23	0.31	>128	64	64	4
	H. influenzae SB1	0.50	0.5	0.25	>128	0.125
	S. aureus 19 MRSA	0.53	128	4	4	2

MBI 11G7CN	*A. calcoaceticus* 3	0.50	128	32	64	16
	S. marcescens 1	0.25	64	16	>128	1
	P. aeruginosa 7	0.50	>128	64	>128	64
	P. aeruginosa 23	0.50	128	64	>128	1
	H. influenzae SB1	0.52	0.5	0.25	>128	4
	S. aureus 18 MRSA	0.50	>128	64	32	8
	S. aureus 19 MRSA	0.56	128	64	8	0.5
MBI 21A2	*E. coli* 1	0.53	>128	8	4	2
	S. maltophilia 6	0.38	>128	64	128	16
	S. maltophilia 14	0.53	128	4	32	16
	S. marcescens 1	0.27	64	16	>128	4
	P. aeruginosa 23	0.19	64	8	>128	16
	H. influenzae 1	0.31	64	4	>128	64
	H. influenzae 2	0.38	128	32	>128	32
	S. aureus 19 MRSA	0.51	128	64	>128	2
	S. aureus SB2MRSA	0.56	128	64	32	2
MBI 26	*S. maltophilia* 3	0.50	128	32	16	4
	S. marcescens 1	0.50	64	32	>128	0.5
	P. aeruginosa 7	0.25	>128	32	>128	32
	P. aeruginosa 41	0.53	64	32	128	4
	H. influenzae 1	0.53	64	32	>128	8
	H. influenzae 2	0.51	128	64	>128	2
	S. aureus 19 MRSA	0.16	128	16	32	1
	S. aureus SB3MRSA	0.31	128	64	>128	16
	A. calcoaceticus 7	0.25	32	4	>32	8
	A. calcoaceticus 8	0.19	64	4	>32	8
	E. cloacae 13	0.16	128	4	>32	8
	P. aeruginosa 23	0.27	256	4	>64	32
	P. aeruginosa 28	0.14	>512	16	>128	32
	S. maltophilia 34	0.25	>512	4	>32	16
	S. maltophilia 35	0.26	>256	4	>32	16

MBI 29	*S. marcescens* 1	0.14	64	32	>128	4
	P. aeruginosa 7	0.53	128	4	16	8
	P. aeruginosa 23	0.50	128	32	16	4
	P. aeruginosa 41	0.56	64	32	64	4
	H. influenzae 1	0.51	32	16	16	0.125
	S. aureus 11 MRSA	0.50	>128	0.5	16	8
	A. calcoaceticus 2	0.50	>512	4	16	8
	A. calcoaceticus 7	0.25	32	4	>16	4
	E. cloacae 16	0.50	>512	4	>16	16
	E. cloacae 17	0.50	>512	4	>16	16
	P. aeruginosa 28	0.13	>512	8	>64	16
	P. aeruginosa 29	0.27	512	8	>32	16
	S. maltophilia 34	0.25	>512	4	>16	8
	S. maltophilia 38	0.28	>512	32	>32	16
	S. maltophilia 40	0.25	>512	4	>32	16
	S. maltophilia 42	0.25	>512	4	>16	8

7. Tobramycin

Peptide	Organism	FIC	Tobramycin MIC (µg/ml)		Peptide MIC (µg/ml)	
			Alone	+ Peptide	Alone	+ Tobramycin
MBI 11A1CN	*P. aeruginosa* PA026	0.50	8	4	>128	0.125
	P. aeruginosa PA032	0.50	16	8	>128	0.5
	S. maltophilia SMA029	0.16	128	4	>128	32
	S. maltophilia SMA030	0.27	128	2	>128	64
	S. aureus SA014	0.50	>128	0.125	16	8
	S. aureus SA025	0.50	>128	0.125	8	4
	S. haemolyticus SHA001	0.52	4	2	8	0.125
	S. haemolyticus SHA005	0.51	8	4	16	0.125
MBI 11B9CN	*A. baumannii* ABI001	0.50	16	4	32	8
	B. cepacia BC002	0.38	>128	64	>128	32
	P. aeruginosa PA008	0.50	32	0.125	128	64
	P. aeruginosa PA025	0.56	32	2	128	64
	S. maltophilia SMA029	0.13	64	4	>128	16
MBI 11CN	*A. baumannii* ABI001	0.50	16	4	64	16
	E. coli ECO006	0.53	8	4	8	0.25
	P. aeruginosa PA032	0.52	16	8	>128	4
	S. maltophilia SMA029	0.51	128	64	>128	2
	S. maltophilia SMA035	0.38	32	4	128	32
MBI 11D18CN	*A. baumannii* ABI001	0.31	16	4	64	4
	A. baumannii ABI002	0.53	8	4	16	0.5
	S. maltophilia SMA027	0.19	32	4	>128	16
	S. maltophilia SMA029	0.16	128	4	32	4
	S. aureus SA018 MRSA	0.56	64	4	32	16
	S. haemolyticus SHA001	0.53	4	0.125	2	1

MBI 11F3CN	*A. baumannii* ABI001	0.53	16	0.5	32	16
	A. baumannii ABI002	1.00	4	2	16	8
	P. aeruginosa PA032	0.50	16	4	>128	64
	S. maltophilia SMA029	0.28	128	32	128	4
	S. maltophilia SMA030	0.26	128	1	128	32
	S. aureus SA014 MRSA	0.51	>128	2	4	2
	S. haemolyticus SHA005	0.56	4	0.25	4	2
MBI 11G13CN	*A. baumannii* ABI001	0.50	16	4	128	32
	P. aeruginosa PA022	0.56	8	4	>128	16
	S. maltophilia SMA029	0.50	128	64	>128	0.125
	S. maltophilia SMA030	0.50	128	64	>128	0.125
	S. aureus SA025 MRSA	0.50	>128	0.125	4	2
MBI 21A1	*B. cepacia* BC001	0.25	128	32	>128	0.25
	P. aeruginosa PA022	0.53	8	4	4	0.125
	P. aeruginosa PA026	0.51	8	4	16	0.125
	S. maltophilia SMA029	0.28	128	4	128	32
	S. maltophilia SMA030	0.16	128	4	>128	32
	S. aureus SA014 MRSA	0.50	>128	0.125	32	16
	S. aureus SA025 MRSA	0.50	>128	0.125	2	1
	S. haemolyticus SHA001	0.50	2	0.5	16	4
	S. haemolyticus SHA005	0.38	4	1	32	4
MBI 22A1	*S. maltophilia* SMA030	0.26	128	1	32	8
	S. maltophilia SMA031	0.25	128	0.5	32	8
	S. aureus SA014 MRSA	0.27	>128	4	8	2
	S. epidermidis SE072	0.50	>128	0.125	16	8
	S. epidermidis SE073	0.50	>128	0.125	16	8
	S. epidermidis SE080	0.56	32	16	2	0.125
MBI 26	*S. maltophilia* SMA029	0.05	128	4	>128	4
	S. maltophilia SMA030	0.05	128	4	>128	4
	S. epidermidis SE067	0.38	>128	64	2	0.25
	S. epidermidis SE068	0.27	>128	4	2	0.5
MBI 27	*E. coli* ECO006	0.56	8	0.5	8	4
	S. maltophilia SMA029	0.50	64	16	16	4
	S. maltophilia SMA031	0.53	128	4	16	8
MBI 29	*A. baumannii* ABI001	0.53	16	8	4	0.125
	E. coli ECO004	0.53	2	1	4	0.125
	E. coli ECO006	0.53	8	4	4	0.125
	K. pneumoniae KP008	0.52	0.5	0.25	8	0.125
	P. aeruginosa PA030	0.52	16	8	8	0.125
	S. maltophilia SMA031	0.50	>128	0.25	16	8
	S. maltophilia SMA032	0.53	128	4	16	8
	S. epidermidis SE072	0.53	>128	8	16	8

MBI 29A3	*P. aeruginosa* PA022	0.56	8	4	4	0.25
	P. aeruginosa PA028	0.50	32	16	32	0.125
	P. aeruginosa PA029	0.51	32	16	16	0.125
	S. maltophilia SMA029	0.28	128	4	16	4
	S. maltophilia SMA030	0.28	128	4	16	4
REWH 53A5CN	*S. maltophilia* SMA029	0.08	128	2	>128	16
	S. maltophilia SMA030	0.13	128	0.25	>128	32
	S. aureus SA014 MRSA	0.50	>128	0.125	16	8

8. Vancomycin

Peptide	Organism	FIC	Vancomycin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Vancomycin
MBI 11A1CN	*E. faecalis* EFS001	0.53	1	0.5	4	0.125
	E. faecalis EFS006	0.50	8	4	128	0.25
	E. faecalis EFS007	0.50	4	2	128	0.5
	E. faecalis EFS010	0.27	16	4	128	2
	E. faecalis EFS012	0.25	>128	32	64	8
	E. faecalis EFS014	0.51	128	1	4	2
	E. faecium EFM004	0.50	>128	0.5	32	16
	E. faecium EFM007	0.28	128	4	64	16
	E. faecium EFM009	0.25	32	4	64	8
MBI 11D18CN	*E. faecalis* EFS001	0.38	1	0.125	8	2
	E. faecalis EFS004	0.50	2	0.5	8	2
	E. faecalis EFS011	0.50	64	32	64	0.125
	E. faecalis EFS012	0.38	>128	64	16	2
	E. faecalis EFS014	0.16	128	4	4	0.5
	E. faecium EFM004	0.50	>128	64	8	2
	E. faecium EFM009	0.52	64	32	8	0.125
	E. faecium EFM010	0.28	>128	64	8	0.25
	E. faecium EFM011	0.50	>128	64	8	2
MBI 21A1	*E. faecalis* EFS007	0.56	2	1	16	1
	E. faecalis EFS012	0.16	128	16	32	1
	E. faecalis EFS013	0.28	128	32	32	1
	E. faecium EFM010	0.56	64	32	32	2
MBI 26	*E. faecalis* EFS005	0.31	16	4	>128	16
	E. faecalis EFS012	0.07	>128	2	16	1
	E. faecalis EFS013	0.07	>128	2	16	1
	E. faecium EFM010	0.31	32	2	32	8
	E. faecium EFM011	0.31	32	2	32	8
	E. faecium EFM012	0.31	32	2	64	16
	E. faecium EFM014	0.27	>128	4	32	8
	E. faecium EFM016	0.51	128	1	8	4

MBI 29	*E. faecalis* EFS005	0.38	16	4	32	4
	E. faecalis EFS010	0.38	64	16	2	0.25
	E. faecalis EFS012	0.50	>128	64	2	0.5
	E. faecium EFM005	0.53	128	4		4
	E. faecium EFM016	0.51	128	1	4	2
MBI 29A3	*E. faecalis* EFS003	0.56	4	2	32	2
	E. faecalis EFS005	0.28	16	4	32	1
	E. faecalis EFS011	0.50	16	4	32	8
	E. faecalis EFS014	0.52	64	1	1	0.5
	E. faecium EFM006	0.52	>128	4	4	2

EXAMPLE 5

OVERCOMING TOLERANCE BY ADMINISTERING A COMBINATION OF ANTIBIOTIC AGENT AND CATIONIC PEPTIDE

Tolerance to an antibiotic agent is associated with a defect in bacterial cellular autolytic enzymes such that an antimicrobial agent is bacteriostatic rather than bactericidal. Tolerance is indicated when a ratio of minimum bactericidal concentration (MBC) to minimum inhibitory concentration (MIC) (MBC:MIC) is ≥ 32.

The agarose dilution assay is adapted to provide both the MBC and MIC for an antimicrobial agent alone and an agent in combination with a peptide. Following determination of MIC, MBC is determined from the agarose dilution assay plates by swabbing the inocula on plates at and above the MIC and resuspending the swab in 1.0 ml of saline. A 0.01 ml aliquot is plated on agarose medium (subculture plates) and the resulting colonies are counted. If the number of colonies is less than 0.1% of the initial inoculum (as determined by a plate count immediately after inoculation of the MIC test plates), then ≥ 99.9% killing has occurred. The MBC end point is defined as the lowest concentration of the antimicrobial agent that kills 99.9% of the test bacteria.

Thus, tolerance of a microorganism to an antimicrobial agent occurs when the number of colonies growing on subculture plates exceeds the 0.1% cutoff for several successive concentrations above the observed MIC. A combination of antimicrobial agent and cationic peptide that breaks tolerance results in a decrease in the MBC:MIC ratio to < 32. Table 13 shows that the combination of Vancomycin and MBI 26 overcomes the tolerance of the organisms listed.

Table 13

Organism	Vancomycin			Vancomycin + MBI 26		
	MIC (μg/ml)	MBC (μg/ml)	MBC/MIC	MIC (μg/ml)	MBC (μg/ml)	MBC/MIC
E. casseliflavus ECA001	2	>128	>64	0.5	2	4
E. faecium EFM001	0.5	>128	>256	0.5	0.5	1
E. faecium EFM020	1	>128	>128	0.5	4	8
E. faecalis EFS001	1	>128	>128	0.5	4	8
E. faecalis EFS004	1	>128	>128	1	2	2
E. faecalis EFS007	4	128	32	2	2	1
E. faecalis EFS009	4	>128	>32	4	4	1
E. faecalis EFS015	1	>128	>128	0.5	0.5	1

EXAMPLE 6

OVERCOMING INHERENT RESISTANCE BY ADMINISTERING A COMBINATION OF ANTIBIOTIC AGENT AND CATIONIC PEPTIDE

Peptides are tested for their ability to overcome the inherent antimicrobial resistance of microorganisms, including those encountered in hospital settings, to specific antimicrobials. Overcoming resistance is demonstrated when the antibiotic agent alone exhibits minimal or no activity against the microorganism, but when used in combination with a cationic peptide, results in susceptibility of the microorganism.

The agarose dilution assay described above is used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and cationic peptides, alone and in combination. Alternatively, the broth dilution assay or time kill curves can be used to determine MICs. Tables 14-17 present MIC values for antibiotic agents alone and in combination with peptide at the concentration shown. In all cases, the microorganism is inherently resistant to its mode of action, thus, the antibiotic agent is not effective against the test microorganism. In addition, the antibiotic agent is not clinically prescribed against the test microorganism.

In the data presented below, the MIC values for the antibiotic agents when administered in combination with peptide are decreased, from equal to or above the resistant breakpoint to below it.

Table 14

	Erythromycin MIC (μg/ml)		MBI 26 MIC (μg/ml)	
Microorganism	Alone	+ MBI 26	Alone	+ Erythro.
A. calcoaceticus AC001	32	1	16	8
K. pneumoniae KP001	32	0.25	16	8
K. pneumoniae KP002	256	0.5	64	32
P. aeruginosa PA041	128	4	64	32

Table 15

Microorganism	Vancomycin MIC (μg/ml)		MBI 26 MIC (μg/ml)	
	Alone	+ MBI 26	Alone	+ Vancomycin
E. gallinarum 97044 VanC	8	2	8	0.5
E. gallinarum 97046 VanC	32	1	2	4
E. gallinarum 97047 VanC	128	16	64	8
E. gallinarum 97048 VanC	32	4	2	2
E. gallinarum 97049 VanC	128	4	64	16
E. casseliflavus 97056 VanC	8	2	8	1
E. casseliflavus 97057 VanC	4	2	2	0.5
E. casseliflavus 97058 VanC	2	1	4	0.25
E. casseliflavus 97059 VanC	4	2	32	0.5
E. casseliflavus 97060 VanC	2	2	0.5	0.25

Table 16

Microorganism	Teicoplanin MIC (μg/ml)		MBI 26 MIC (μg/ml)	
	Alone	+ MBI 26	Alone	+ Vancomycin
E. gallinarum 97044 VanC	0.5	0.25	64	1
E. gallinarum 97046 VanC	1	0.25	8	1
E. gallinarum 97047 VanC	8	0.25	64	32
E. gallinarum 97048 VanC	0.5	0.25	8	1
E. gallinarum 97049 VanC	2	0.25	64	32
E. casseliflavus 97056 VanC	0.5	0.25	64	2
E. casseliflavus 97057 VanC	0.5	0.25	64	0.5
E. casseliflavus 97058 VanC	0.5	0.25	32	0.5
E. casseliflavus 97059 VanC	0.5	0.25	64	1
E. casseliflavus 97060 VanC	0.5	0.25	64	1

Table 17

1. Amikacin

Peptide	Organism	FIC	Amikacin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Amikacin
MBI 11B16CN	*A. baumannii* ABI001	0.25	32	4	32	4
	S. maltophilia SMA018	0.31	128	8	32	8
	S. maltophilia SMA022	0.14	>128	4	>128	32
	S. aureus SA014 MRSA	0.75	32	8	8	4
	S. aureus SA025 MRSA	0.63	32	4	8	4
MBI 21A2	*S. maltophilia* SMA018	0.53	>128	8	16	8
	S. maltophilia SMA060	0.31	>128	16	>128	64
	S. aureus SA025 MRSA	0.56	32	2	2	1
MBI 26	*S. maltophilia* SMA022	0.19	128	8	64	8
	S. maltophilia SMA037	0.19	128	16	>128	16
MBI 27	*A. baumannii* ABI001	1.00	32	16	8	4
	B. cepacia BC005	0.50	64	16	>128	64
	S. maltophilia SMA036	0.56	>128	16	64	32
	S. maltophilia SMA037	0.31	64	4	64	16
	S. aureus SA025 MRSA	0.75	32	16	2	0.5
MBI 29A3	*B. cepacia* BC003	0.63	32	16	>128	32
	B. cepacia BC005	0.38	128	32	>128	32
	S. maltophilia SMA036	0.53	>128	8	64	32
	S. maltophilia SMA063	0.56	>128	16	8	4
MBI 29F1	*A. baumannii* ABI001	0.75	32	16	8	2
	S. maltophilia SMA018	0.56	128	8	4	2
	S. maltophilia SMA021	0.31	128	8	8	2
	S. aureus SA014 MRSA	0.53	32	16	4	0.125
	S. aureus SA025 MRSA	0.63	32	16	1	0.125
Deber A2KA2	*A. baumannii* ABI001	0.63	32	16	>128	32
	S. aureus SA025 MRSA	0.50	32	0.125	16	8

2. Ceftriaxone

Peptide	Organism	FIC	Ceftriaxone MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Ceftriaxone
MBI 11B7CN	*P. aeruginosa* PA008	0.50	128	0.125	128	64
	S. maltophilia SMA021	0.50	>128	1	32	16
	S. maltophilia SMA023	0.56	128	8	128	64
MBI 11J02CN	*P. aeruginosa* PA008	0.50	64	0.125	64	32
	P. aeruginosa PA039	0.52	64	1	64	32
MBI 26	*P. aeruginosa* PA008	0.13	64	8	128	0.125
	P. aeruginosa PA024	0.50	16	4	128	32
	S. maltophilia SMA021	0.25	>128	1	8	2

3. Gentamicin

Peptide	Organism	FIC	Gentamicin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Gentamicin
MBI 11B16CN	*S. aureus* SA014 MRSA	0.53	32	1	8	4
MBI 27	*S. aureus* SA014 MRSA	0.50	32	0.125	2	1

4. Mupirocin

Peptide	Organism	FIC	Mupirocin MIC (µg/ml)		Peptide MIC (µg/ml)	
			Alone	+ Peptide	Alone	+ Mupirocin
MBI 11B4CN	*E. coli* ECO3	0.53	100	3	16	8
MBI 11D18CN	*E. coli* ECO3	0.26	100	1	4	1
MBI 21A1	*E. coli* ECO1	0.50	>100	3	2	1
	E. coli ECO2	0.53	100	3	2	1
	E. coli ECO3	0.28	100	3	8	2
MBI 26	*E. coli* ECO1	0.50	>100	3	2	1
MBI 27	*P. aeruginosa* PA2	0.25	>100	0.1	64	16
	P. aeruginosa PA4	0.50	>100	0.3	32	16
MBI 28	*E. coli* SBECO1	0.50	100	0.1	4	2
	P. aeruginosa PA4	0.50	>100	3	32	16
MBI 29A3	*P. aeruginosa* SBPA2	0.50	>100	0.1	16	8
	P. aeruginosa PA2	0.50	>100	0.1	32	16
	P. aeruginosa PA3	0.50	>100	0.1	16	8
	P. aeruginosa PA4	0.50	>100	0.1	16	8

5. Piperacillin

Peptide	Organism	FIC	Piperacillin MIC (µg/ml)		Peptide MIC (µg/ml)	
			Alone	+ Peptide	Alone	+ Piperacillin
MBI 11B7CN	*S. aureus* 19 MRSA	0.50	128	0.5	4	2
MBI 11D18CN	*S. aureus* 19 MRSA	0.52	128	2	4	2
MBI 11E3CN	*S. aureus* 19 MRSA	0.51	>128	2	4	2
MBI 11F3CN	*S. aureus* 19 MRSA	0.51	>128	2	4	2
	S. aureus SB3MRSA	0.52	>128	4	8	4
MBI 11F4CN	*S. aureus* 19 MRSA	0.53	128	4	4	2
MBI 11G7CN	*S. aureus* 19 MRSA	0.25	128	0.5	8	2
MBI 21A2	*S. aureus* 19 MRSA	0.25	128	0.5	>128	64
MBI 26	*S. aureus* 19 MRSA	0.13	128	0.5	32	4
MBI 29	*S. aureus* 18 MRSA	0.52	>128	4	16	8

6. Tobramycin

Peptide	Organism	FIC	Tobramycin MIC (µg/ml)		Peptide MIC (µg/ml)	
			Alone	+ Peptide	Alone	+ Tobramycin
MBI 11A1CN	*S. aureus* SA014	0.50	>128	0.125	16	8
	S. aureus SA025	0.50	>128	0.125	8	4
	S. haemolyticus SHA005	0.51	8	4	16	0.125
MBI 11D18CN	*S. aureus* SA018 MRSA	0.56	64	4	32	16
MBI 11F3CN	*S. aureus* SA014 MRSA	0.51	>128	2	4	2
MBI 11G13CN	*S. aureus* SA025 MRSA	0.50	>128	0.125	4	2
MBI 21A1	*S. aureus* SA014 MRSA	0.50	>128	0.125	32	16
	S. aureus SA025 MRSA	0.50	>128	0.125	2	1
MBI 22A1	*S. aureus* SA014 MRSA	0.27	>128	4	8	2

EXAMPLE 7

OVERCOMING ACQUIRED RESISTANCE BY ADMINISTERING A COMBINATION OF ANTIBIOTIC AGENT AND CATIONIC PEPTIDE

An antibiotic agent can become ineffective against a previously susceptible microorganism if the microorganism acquires resistance to the agent. However, acquired resistance can be overcome when the agent is administered in combination with a cationic peptide. For example vancomycin resistant enterococci (VRE) become susceptible to vancomycin when it is used in combination with a cationic peptide such as MBI 26. This combination is likely to be effective against other organisms acquiring resistance to vancomycin including but not limited to strains of methicillin resistant S. aureus (MRSA).

Similarly teicoplanin resistant enterococci become susceptible to teicoplanin when teicoplanin is used in combination with cationic peptides such as MBI 26.

As described previously, the agarose dilution assay is used to determine the MIC for antibiotic agents administered alone and in combination with cationic peptide. Alternatively the broth dilution assay or time kill curves can be employed. Tables 18 and 19 presents results showing that administration of a cationic peptide in combination with an antibiotic agent overcomes acquired resistance. Table 20 presents results showing administration of MBI 26 in combination with teicoplanin against teicoplanin resistant enterococci.

Table 18

Microorganism	Strain	Antibiotic agent	MIC alone (µg/ml)	MIC comb. (µg/ml)	Peptide	Peptide MIC
A. calcoaceticus	002	Tobramycin	8	1	MBI 29	4
A. calcoaceticus	003	Ceftazidime	32	2	MBI 26	32
A. calcoaceticus	003	Ceftazidime	32	2	MBI 29	8
A. calcoaceticus	003	Ciprofloxacin	8	1	MBI 29	16
A. calcoaceticus	004	Ciprofloxacin	8	4	MBI 26	4
A. calcoaceticus	010	Ceftazidime	32	2	MBI 26	32
E. faecium	ATCC 29212	Mupirocin	100	0.1	MBI 11CN	8
E. faecium	ATCC 29212	Mupirocin	100	0.1	MBI 11G13CN	32
P. aeruginosa	PA41	Ciprofloxacin	4	0.125	MBI 21A1	16
P. aeruginosa	PA41	Ciprofloxacin	4	1	MBI 21A2	16
P. aeruginosa	PA41	Ciprofloxacin	8	2	MBI 28	8
P. aeruginosa	001	Piperacillin	128	64	MBI 27	8
P. aeruginosa	023	Piperacillin	128	64	MBI 29	8
P. aeruginosa	024	Tobramycin	64	1	MBI 29	8
P. aeruginosa	025	Ceftazidime	64	16	MBI 29	8

Microorganism	Strain	Antibiotic agent	MIC alone (µg/ml)	MIC comb. (µg/ml)	Peptide	Peptide MIC
P. aeruginosa	027	Imipenem	16	8	MBI 29	16
P. aeruginosa	028	Imipenem	16	8	MBI 29	16
S,. haemolyticus	SH8578	Erythromycin	8	0.5	MBI 31	1
S. aureus	SA7338	Ampicillin	2	0.25	MBI 26	0.25
S. aureus	SA7609	Erythromycin	32	0.5	MBI 26	1
S. aureus	SA7835	Erythromycin	8	0.125	MBI 26	2
S. aureus	SA7795	Erythromycin	32	1	MBI 26	8
S. aureus	SA7796	Erythromycin	32	1	MBI 26	2
S. aureus	SA7795	Erythromycin	32	4	MBI 31	0.125
S. aureus	SA7818	Erythromycin	32	2	MBI 31	0.125
S. aureus	SA7796	Erythromycin	32	2	MBI 31	0.125
S. aureus	SA7834	Methicillin	32	8	MBI 26	4
S. aureus	SA7835	Methicillin	32	4	MBI 26	16
S. aureus	SA7796	Methicillin	16	2	MBI 31	16
S. aureus	SA7797	Methicillin	16	2	MBI 31	16
S. aureus	SA7823	Methicillin	16	2	MBI 31	0.5
S. aureus	SA7834	Methicillin	64	1	MBI 31	32
S. aureus	SA7835	Methicillin	64	2	MBI 31	16
S. aureus	SA007	Piperacillin	128	64	MBI 27	0.5
S. aureus	MRSA 9	Mupirocin	>100	0.1	MBI 11D18CN	2
S. aureus	MRSA 9	Mupirocin	>100	0.1	MBI 11G13CN	8
S. aureus	MRSA 9	Mupirocin	>100	0.1	MBI 21A1	16
S. aureus	MRSA 9	Mupirocin	>100	0.3	MBI 21A10	32
S. aureus	MRSA 9	Mupirocin	>100	0.1	MBI 21A2	32
S. aureus	MRSA 9	Mupirocin	>100	0.1	MBI 26	4
S. aureus	MRSA 9	Mupirocin	>100	0.1	MBI 27	2
S. aureus	MRSA 13	Mupirocin	100	3	MBI 10CN	4
S. aureus	MRSA 13	Mupirocin	100	0.1	MBI 11CN	16
S. aureus	MRSA 13	Mupirocin	100	3	MBI 11F1CN	8
S. aureus	014	Ciprofloxacin	8	0.125	MBI 21A2	4
S. aureus	MRSA 17	Mupirocin	>100	1 0.3	MBI 10CN	1 2
S. aureus	MRSA 17	Mupirocin	>100	1	MBI 11A1CN	32
S. aureus	MRSA 17	Mupirocin	>100	1	MBI11G13CN	16
S. aureus	MRSA 17	Mupirocin	>100	0.3	MBI 27	2
S. aureus	MRSA 17	Mupirocin	>100	0.1	MBI 29A3	4.
S. aureus	093	Ciprofloxacin	32	0.125	MBI 21A1	2
S. aureus	093	Ciprofloxacin	32	1	MBI 21A2	4
S. aureus	SA 7818	Methicillin	16	4	MBI 26	2
S. epidermidis	SE8497	Clindamycin	32	0.125	MBI 26	2
S. epidermidis	SE8403	Erythromycin	8	0.125	MBI 26	2
S. epidermidis	SE8410	Erythromycin	32	0.5	MBI 26	1
S. epidermidis	SE8411	Erythromycin	32	0.5	MBI 26	1
S. epidermidis	SE8497	Erythromycin	32	0.125	MBI 26	1
S. epidermidis	SE8503	Erythromycin	32	0.5	MBI 26	1
S. epidermidis	SE8565	Erythromycin	32	0.5	MBI 26	1
S. epidermidis	SE8403	Erythromycin	8	0.125	MBI 31	2
S. epidermidis	SE8410	Erythromycin	32	0.5	MBI 31	1
S. epidermidis	SE8411	Erythromycin	32	0.5	MBI 31	1
S. epidermidis	SE8497	Erythromycin	32	0.125	MBI 31	1

Microorganism	Strain	Antibiotic agent	MIC alone (μg/ml)	MIC comb. (μg/ml)	Peptide	Peptide MIC
S. epidermidis	SE8503	Erythromycin	32	0.5	MBI 31	1
S. epidermidis	SE8565	Erythromycin	32	0.5	MBI 31	1
S. haemolyticus	SH8459	Ampicillin	0.5	0.25	MBI 26	0.25
S. haemolyticus	SH8472	Ampicillin	2	0.25	MBI 26	16
S. haemolyticus	SH8564	Ampicillin	64	0.25	MBI 26	32
S. haemolyticus	SH8575	Ampicillin	0.5	0.25	MBI 26	8
S. haemolyticus	SH8578	Ampicillin	0.5	0.25	MBI 26	4
S. haemolyticus	SH8597	Clindamycin	16	0.125	MBI 26	1
S. haemolyticus	SH8463	Erythromycin	8	0.5	MBI 26	0.5
S. haemolyticus	SH8472	Erythromycin	8	0.5	MBI 26	0.5
S. haemolyticus	SH8575	Erythromycin	32	2	MBI 26	0.5
S. haemolyticus	SH8578	Erythromycin	8	0.5	MBI 26	01
S. haemolyticus	SH8597	Erythromycin	32	0.5	MBI 26	0.5
S. haemolyticus	SH8463	Erythromycin	8	0.5	MBI 31	0.5
S. haemolyticus	SH8472	Erythromycin	8	0.5	MBI 31	0.5
S. haemolyticus	SH8564	Erythromycin	32	2	MBI 31	0.5
S. haemolyticus	SH8575	Erythromycin	32	2	MBI 31	0.5
S. haemolyticus	SH8563	Methicillin	64	0.25	MBI 26	2
S. maltophilia	034	Tobramycin	8	1	MBI 29	4
S. maltophilia	037	Tobramycin	32	4	MBI 29	16
S. maltophilia	039	Ciprofloxacin	4	2	MBI 29	16
S. maltophilia	041	Tobramycin	16	1	MBI 29	8
S. maltophilia	043	Imipenem	>256	4	MBI 29	16
S. maltophilia	044	Piperacillin	>512	16	MBI 26	32

Table 19

Microorganism	Strain	Teicoplanin (μg/ml)		MBI 26 (μg/ml)	
		Alone	+ MBI 26	Alone	+ Teicoplanin
E. faecium 97017	VanA	32	0.25	64	4
E. faecium 97018	VanA	32	0.25	64	8
E. faecium 97019	VanA	32	0.5	64	16
E. faecium 97020	VanA	32	0.5	64	16
E. faecium 97021	VanA	32	0.5	64	32
E. faecium 97022	VanA	32	0.5	64	4
E. faecium 97023	VanA	32	0.25	64	4
E. faecium 97024	VanA	32	0.25	64	8
E. faecium 97025	VanA	32	0.5	16	4
E. faecium 97026	VanA	32	0.5	64	16
E. faecium 97027	VanA	32	8	64	8
E. faecium 97028	VanA	32	0.25	8	8
E. faecium 97029	VanA	32	0.25	64	8
E. faecium 97030	VanA	32	0.25	64	32
E. faecium 97031	VanA	32	0.25	64	32
E. faecium 97032	VanA	32	0.25	64	8
E. faecium 97033	VanA	32	0.25	64	8
E. faecium 97034	VanA	32	0.25	64	8
E. faecium 97035	VanA	32	0.25	64	0.5
E. faecium 97036	VanA	8	0.25	8	4
E. faecalis 97050	VanA	32	0.25	64	8
E. faecalis 97051	VanA	32	0.25	64	8
E. faecalis 97052	VanA	32	0.25	64	8
E. faecalis 97053	VanA	32	0.25	64	8
E. faecalis 97054	VanA	32	0.25	64	8
E. faecalis 97055	VanA	32	0.25	64	8

Table 20

1. Amikacin

Peptide	Organism	FIC	Amikacin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Amikacin
MBI 11B16CN	*P. aeruginosa* PA022	0.38	64	8	64	16
MBI 21A2	*P. aeruginosa* PA022	0.50	64	16	8	2
	E. faecium EFM020	0.56	32	2	128	64
	E. faecalis EFS008	0.19	64	8	>128	16
MBI 26	*E. faecium* EFM004	0.56	128	8	64	32
	E. faecium EFM020	0.75	32	8	64	32
MBI 27	*E. faecium* EFM004	0.75	64	16	16	8
	E. faecium EFM020	0.63	32	4	16	8
	E. faecalis EFS008	0.56	32	16	4	0.25
MBI 29A3	*E. faecium* EFM004	0.56	128	8	8	4
	E. faecium EFM020	1.00	32	16	4	2
MBI 29F1	*E. faecium* EFM004	0.53	>128	8	32	16
	E. faecalis EFS008	0.19	64	4	4	0.5
Deber A2KA2	*E. faecalis* EFS008	0.19	64	8	>128	16

2. Ceftriaxone

Peptide	Organism	FIC	Ceftriaxone MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Ceftriaxone
MBI 11B7CN	*A. baumannii* ABI002	0.50	32	8	32	8
	A. baumannii ABI005	0.56	16	8	16	1
MBI 11J02CN	*A. baumannii* ABI005	0.56	16	8	8	0.5
	A. lwoffii ALW007	0.75	16	4	4	2
	B. cepacia BC003	0.63	16	8	>128	32
	E. cloacae ECL014	0.50	128	0.25	32	16
	E. cloacae ECL015	0.52	64	1	32	16
MBI 26	*A. baumannii* ABI005	0.53	16	0.5	2	1
	A. baumannii ABI006	0.56	128	8	2	1
	B. cepacia BC003	0.50	16	8	>128	0.125
	E. cloacae ECL015	0.19	64	4	32	4

3. Ciprofloxacin

Peptide	Organism	FIC	Ciprofloxacin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Ciprofloxacin
MBI 11A1CN	*S. aureus* SA10	0.50	32	0.125	128	64
	S. aureus SA25	0.53	4	0.125	16	8
MBI 11D18CN	*P. aeruginosa* PA77	0.50	2	0.5	128	32
MBI 21A1	*S. aureus* SA25	0.16	4	0.125	32	4
	P. aeruginosa PA41	0.50	4	1	16	4
	P. aeruginosa PA77	1.00	2	1	32	16
MBI 21A2	*S. aureus* SA25	0.56	2	1	16	1
	P. aeruginosa PA41	0.50	4	1	64	16
	P. aeruginosa PA77	0.63	2	0.25	64	32

MBI 26	*A. calcoaceticus* 5	0.38	2	0.25	>32	16
	E. cloacae 16	0.38	2	0.25	>32	16
	E. cloacae 17	0.38	2	0.25	>32	16
	P. aeruginosa PA41	0.50	4	1	128	32
	P. aeruginosa PA77	0.56	2	0.125	128	64
	P. aeruginosa 30	0.09	4	0.25	>32	2
	P. aeruginosa 31	0.27	16	0.25	>32	16
	S. maltophilia 34	0.25	2	0.25	>32	8
	S. maltophilia 35	0.50	2	0.5	>32	16
MBI 27	*S. aureus* SA25	0.75	4	1	2	1
MBI 28	*S. aureus* SA25	0.56	2	0.125	2	1
MBI 29	*A. calcoaceticus* 3	0.63	8	1	>16	16
	A. calcoaceticus 4	0.63	8	1	>16	16
	E. cloacae 16	0.63	2	0.25	>16	16
	E. cloacae 17	0.75	2	1	16	4
	S. aureus SA10	0.50	32	0.125	4	2
	S. aureus SA14	0.63	8	1	8	4
	P. aeruginosa PA41	0.63	8	1	8	4
	P. aeruginosa PA77	0.50	2	0.5	64	16
	P. aeruginosa 30	0.56	4	0.25	>16	16
	P. aeruginosa 31	0.53	16	0.5	>16	16
	S. maltophilia 34	0.63	2	0.25	>16	16
	S. maltophilia 35	0.63	2	0.25	>16	16
MBI 29A2	*S. aureus* SA10	0.52	32	0.5	4	2
	S. aureus SA25	0.63	4	0.5	2	1
	P. aeruginosa PA41	1.00	4	2	8	4
	P. aeruginosa PA77	1.00	2	1	16	8
MBI 29A3	*S. aureus* SA25	0.75	4	1	1	0.5
	P. aeruginosa PA41	0.63	4	0.5	8	4

4. Gentamicin

Peptide	Organism	FIC	Gentamicin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Gentamicin
MBI 11B16CN	*A. baumannii* ABI001	0.31	64	4	16	4
	A. baumannii ABI002	0.31	32	2	16	4
	A. calcoaceticus AC001	0.25	8	1	32	4
	P. aeruginosa PA023	0.56	8	4	>128	16
	P. aeruginosa PA041	0.31	8	2	>128	16
	S. maltophilia SMA017	0.16	64	2	128	16
	S. maltophilia SMA019	0.51	64	0.5	32	16
MBI 21A2	*A. calcoaceticus* AC001	1.00	8	4	16	8
	P. aeruginosa PA022	0.56	32	2	8	4
	S. maltophilia SMA020	0.50	64	0.125	16	8
	S. maltophilia SMA021	0.50	64	0.125	16	8

MBI 26	*A. baumannii* ABI001	0.56	64	4	8	4
	A. baumannii ABI002	0.53	16	0.5	8	4
	P. aeruginosa PA023	0.75	8	4	>128	64
	P. aeruginosa PA041	0.75	8	4	64	16
	S. maltophilia SMA017	0.52	64	1	16	8
	S. maltophilia SMA019	0.53	64	2	4	2
MBI 27	*A. baumannii* ABI002	0.52	32	0.5	8	4
	A. calcoaceticus AC001	0.63	8	1	8	4
	P. aeruginosa PA023	0.50	16	4	32	8
	P. aeruginosa PA041	1.00	8	4	16	8
	S. maltophilia SMA019	0.50	64	0.125	8	4
	S. maltophilia SMA020	0.50	64	0.125	8	4
MBI 29A3	*A. baumannii* ABI002	0.75	16	4	2	1
	P. aeruginosa PA041	1.00	8	4	8	4
MBI 29F1	*A. calcoaceticus* AC001	0.75	8	2	8	4
	P. aeruginosa PA023	0.52	8	0.125	128	64
Deber A2KA2	*A. calcoaceticus* AC001	0.56	8	4	>128	16
	P. aeruginosa PA041	0.50	16	4	>128	64

5. Mupirocin

Peptide	Organism	FIC	Mupirocin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Mupirocin
MBI 27	*S. aureus* SBSA4	0.50	>100	0.3	4	2

6. Piperacillin

Peptide	Organism	FIC	Piperacillin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Psiperacillin
MBI 11B7CN	*S. maltophilia* 2	1.00	32	16	128	8
	S. marcescens 1	0.27	32	8	>128	4
	H. influenzae 1	0.13	64	8	>128	1
MBI 11B9CN	*A. calcoaceticus* 3	0.75	64	16	32	16
	S. maltophilia 2	0.75	64	16	32	16
	S. marcescens SB1	0.26	64	16	>128	2
	P. aeruginosa 12	0.75	>128	64	128	64
	P. aeruginosa 15	0.50	>128	64	>128	64
MBI 11CN	*A. calcoaceticus* 3	1.00	32	16	64	32
	S. maltophilia 2	0.75	64	16	64	32
	P. aeruginosa 22	0.52	>128	4	64	32
	P. aeruginosa 23	0.53	128	64	128	4
MBI 11D18CN	*A. calcoaceticus* 3	0.38	64	8	32	8
	E. cloacae 9	0.31	>128	16	64	16
	E. cloacae 10	0.56	>128	16	32	16
	S. maltophilia 2	0.50	64	16	32	8
	S. maltophilia 14	0.63	128	16	16	8
	S. marcescens 1	0.14	64	8	>128	4
	P. aeruginosa 23	0.56	128	64	64	4

MBI 11E3CN	*A. calcoaceticus* 3	0.75	32	16	32	8
	S. maltophilia 3	0.75	64	16	32	16
	S. maltophilia 4	0.75	64	16	32	16
	S. marcescens SB1	0.26	64	16	>128	2
	P. aeruginosa 7	1.00	128	64	64	32
	P. aeruginosa 23	0.27	128	32	64	1
	H. influenzae 1	0.38	64	8	>128	64
	H. influenzae 2	0.31	32	8	>128	16
MBI 11F3CN	*A. calcoaceticus* 3	0.63	32	16	32	4
	S. maltophilia 2	0.75	64	16	32	16
	P. aeruginosa 7	1.00	128	64	128	64
	P. aeruginosa 23	0.51	128	64	64	0.5
MBI 11F4CN	*E. cloacae* 10	0.52	>128	4	16	8
	S. maltophilia 2	0.50	64	16	16	4
	S. marcescens 1	0.08	>128	16	>128	4
	P. aeruginosa 7	0.38	>128	64	64	8
	P. aeruginosa 23	0.31	>128	64	64	4
	H. influenzae 1	0.75	32	16	>128	64
MBI 11G7CN	*A. calcoaceticus* 3	0.63	128	16	64	32
	S. maltophilia 2	0.75	64	16	64	16
	S. marcescens 1	0.25	64	16	>128	1
	P. aeruginosa 7	0.50	>128	64	>128	64
	P. aeruginosa 23	0.50	128	64	>128	1
	H. influenzae 1	0.75	32	16	>128	64
MBI 21A2	*E. coli* 1	0.53	>128	8	4	2
	S. maltophilia 3	0.75	64	16	32	16
	S. maltophilia 11	0.75	32	8	128	64
	S. marcescens 1	0.27	64	16	>128	4
	H. influenzae 1	0.31	64	4	>128	64
	H. influenzae 2	0.28	128	4	>128	64
MBI 26	*S. maltophilia* 2	0.75	64	16	4	2
	S. maltophilia 4	0.63	128	16	16	8
	S. marcescens 1	0.09	64	2	>128	16
	P. aeruginosa 7	0.25	>128	32	>128	32
	H. influenzae 1	0.19	64	4	>128	32
	H. influenzae 2	0.19	128	16	>128	16
	A. calcoaceticus 2	0.50	>512	4	32	16
	A. calcoaceticus 7	0.25	32	4	>32	8
	E. cloacae 13	0.16	128	4	>32	8
	E. cloacae 19	0.31	64	4	>32	16
	P. aeruginosa 23	0.27	256	4	>64	32
	P. aeruginosa 26	0.56	128	8	>32	32
	S. maltophilia 35	0.26	>256	4	>32	16
	S. maltophilia 41	0.52	>512	16	>32	32

MBI 29	*S. marcescens* 1	0.09	64	16	>128	8
	P. aeruginosa 23	0.63	128	64	16	2
	H. influenzae 1	0.51	32	16	16	0.125
	A. calcoaceticus 2	0.50	>512	4	16	8
	A. calcoaceticus 7	0.25	32	4	>16	4
	E. cloacae 16	0.50	>512	4	>16	16
	E. cloacae 17	0.50	>512	4	>16	16
	P. aeruginosa 23	0.63	128	64	>32	8
	P. aeruginosa 24	0.50	>512	4	>16	16
	S. maltophilia 34	0.25	>512	4	>16	8
	S. maltophilia 35	0.50	>512	4	>16	16

7. Tobramycin

Peptide	Organism	FIC	Tobramycin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Tobramycin
MBI 11A1CN	*P. aeruginosa* PA026	0.50	8	4	>128	0.125
	S. maltophilia SMA029	0.16	128	4	>128	32
	S. maltophilia SMA030	0.27	128	2	>128	64
MBI 11B9CN	*A. baumannii* ABI001	0.50	16	4	32	8
	E. coli ECO006	0.75	8	4	32	8
	P. aeruginosa PA008	0.50	32	0.125	128	64
	P. aeruginosa PA025	0.56	32	2	128	64
	S. maltophilia SMA027	0.63	8	4	>128	32
	S. maltophilia SMA031	0.19	64	4	>128	32
MBI 11CN	*A. baumannii* ABI001	0.50	16	4	64	16
	E. coli ECO006	0.53	8	4	8	0.25
	P. aeruginosa PA032	0.50	16	4	>128	64
	S. maltophilia SMA029	0.27	128	2	>128	64
	S. maltophilia SMA030	0.27	128	2	>128	64
MBI 11D18CN	*A. baumannii* ABI001	0.31	16	4	64	4
	A. baumannii ABI002	0.53	8	4	16	0.5
	P. aeruginosa PA032	1.00	8	4	64	32
	S. maltophilia SMA027	0.19	32	4	>128	16
	S. maltophilia SMA029	0.27	128	2	32	8
	S. epidermidis SE080	0.75	16	4	2	1
MBI 11F3CN	*A. baumannii* ABI001	0.53	16	0.5	32	16
	P. aeruginosa PA032	0.50	16	4	>128	64
	S. maltophilia SMA029	0.26	128	1	128	32
	S. maltophilia SMA030	0.26	128	1	128	32
MBI 11G13CN	*A. baumannii* ABI001	0.50	16	4	128	32
	P. aeruginosa PA022	0.56	8	4	>128	16
MBI 21A1	*P. aeruginosa* PA022	0.53	8	4	4	0.125
	P. aeruginosa PA026	0.51	8	4	16	0.125
	P. aeruginosa PA030	0.52	16	0.25	16	8
	P. aeruginosa PA032	0.63	8	1	64	32
	S. maltophilia SMA029	0.28	128	4	128	32
	S. maltophilia SMA030	0.16	128	4	>128	32
MBI 22A1	*A. baumannii* ABI001	0.75	16	4	4	2
	S. maltophilia SMA029	0.51	128	1	16	8
	S. maltophilia SMA029	0.50	128	0.125	32	16
	S. epidermidis SE072	0.50	>128	0.125	16	8
	S. epidermidis SE073	0.50	>128	0.125	16	8

MBI 26	*P. aeruginosa* PA031	0.75	16	4	32	16
	S. maltophilia SMA027	0.50	16	4	>128	64
	S. epidermidis SE068	0.27	>128	4	2	0.5
	S. epidermidis SE071	0.50	>128	0.125	16	8
MBI 27	*E. coli* ECO006	0.56	8	0.5	8	4
	S. maltophilia SMA027	1.00	8	4	32	16
	S. maltophilia SMA031	0.53	128	4	16	8
MBI 29	*E. coli* ECO006	0.53	8	4	4	0.125
	P. aeruginosa PA032	1.00	8	4	128	64
	S. maltophilia SMA031	0.50	>128	0.25	16	8
	S. maltophilia SMA032	0.53	128	4	16	8
MBI 29A3	*E. coli* ECO006	0.75	8	2	4	2
	P. aeruginosa PA022	0.56	8	4	4	0.25
	S. maltophilia SMA027	0.75	16	4	32	16
	S. maltophilia SMA029	0.28	128	4	16	4
REWH 53A5CN	*S. maltophilia* SMA029	0.13	128	0.25	>128	32
	S. maltophilia SMA030	0.13	128	0.25	>128	32

8. Vancomycin

Peptide	Organism	FIC	Vancomycin MIC (μg/ml)		Peptide MIC (μg/ml)	
			Alone	+ Peptide	Alone	+ Vancomycin
MBI 11A1CN	*E. faecalis* EFS003	0.63	8	4	>128	32
	E. faecalis EFS006	0.50	8	4	128	0.25
	E. faecalis EFS010	0.13	16	1	128	8
	E. faecalis EFS014	0.51	128	1	4	2
	E. faecium EFM004	0.50	>128	0.5	32	16
	E. faecium EFM007	0.28	128	4	64	16
	E. faecium EFM009	0.25	32	4	64	8
MBI 11D18CN	*E. faecalis* EFS003	0.75	8	2	64	32
	E. faecalis EFS007	0.63	8	1	16	8
	E. faecalis EFS009	0.75	8	2	8	4
	E. faecium EFM004	0.50	>128	0.5	8	4
	E. faecium EFM007	0.50	>128	0.5	8	4
	E. faecium EFM009	0.52	64	1	8	4
	E. faecium EFM010	0.50	>128	1	8	4
MBI 21A1	*E. faecalis* EFS012	0.09	128	4	32	2
	E. faecalis EFS013	0.09	128	4	32	2
	E. faecium EFM010	0.56	64	4	32	16

MBI 26	*E. faecalis* EFS005	0.31	16	4	>128	16
	E. faecalis EFS010	0.27	64	1	4	1
	E. faecalis EFS011	0.25	16	2	>128	32
	E. faecium EFM004	0.25	>128	0.125	64	16
	E. faecium EFM010	0.53	128	1	32	16
	E. faecium EFM011	0.31	32	2	32	8
MBI 29	*E. faecalis* EFS012	0.50	>128	1	2	1
	E. faecalis EFS013	0.50	>128	1	2	1
	E. faecium EFM005	0.53	128	4	8	4
	E. faecium EFM009	0.75	16	4	8	4
	E. faecium EFM010	0.63	32	4	8	4
	E. faecium EFM016	0.51	128	1	4	2
MBI 29A3	*E. faecalis* EFS005	0.19	16	1	32	4
	E. faecalis EFS011	0.50	16	4	32	8
	E. faecalis EFS014	0.52	64	1	1	0.5
	E. faecium EFM006	0.52	>128	4	4	2

These data show that acquired resistance can be overcome. For example, the acquired resistance of *S. aureus*, a Gram-positive organism, to piperacillin is overcome when it is combined with MBI 27 and acquired resistance to ciprofloxacin is overcome with peptides MBI 21A1 or MBI 21A2. Similar results are obtained for peptides MBI 26 and MBI 31 in combination with methicillin and erythromycin, and for peptide MBI 26 in combination with vancomycin or teicoplanin against resistant enterococci.

EXAMPLE 8

SYNERGY OF CATIONIC PEPTIDES AND LYSOZYME OR NISIN

The effectiveness of lysozyme or nisin is improved when either agent is administered in combination with an antibiotic agent. The improvement is demonstrated by measurement of the MICs of lysozyme or nisin alone and in combination with the antibiotic, whereby the lysozyme or nisin, or antibiotic, MIC is lower in combination than alone. The MICs can be measured by the agarose dilution assay, the broth dilution assay or by time kill curves.

EXAMPLE 9

ERYTHROCYTE HEMOLYSIS BY CATIONIC PEPTIDES

A red blood cell (RBC) lysis assay is used to group peptides according to their ability to lyse RBC under standardized conditions compared with MBI 11CN and

Gramicidin-S. Peptide samples and washed sheep RBC are prepared in isotonic saline with the final pH adjusted to between 6 and 7. Peptide samples and RBC suspension are mixed together to yield solutions that are 1% (v/v) RBC and 5, 50 or 500 μg/ml peptide. The assay is performed as described above. Each set of assays also includes MBI 11CN (500 μg/ml) and Gramicidin-S (5 μg/ml) as "low lysis" and "high lysis" controls. respectively.

MBI11B7CN, MBI11F3CN and MBI11F4CN are tested using this procedure and the results are presented in Table 21 below.

Table 21

Peptide	% lysis at 5 μg/ml	% lysis at 50 μg/ml	% lysis at 500 μg/ml
MBI 11B7CN	4	13	46
MBI 11F3CN	1	6	17
MBI 11F4CN	4	32	38
MBI 11CN	N/D	N/D	9
Gramicidin-S	30	N/D	N/D

N/D = not done

Peptides that at 5 μg/ml lyse RBC to an equal or greater extent than Gramicidin-S, the "high lysis" control, are considered to be highly lytic. Peptides that at 500 μg/ml lyse RBC to an equal to or lesser extent than MBI 11CN, the "low lysis" control, are considered to be non-lytic. The three analogues tested are all "moderately lytic" as they cause more lysis than MBI 11CN and less than Gramicidin S. In addition one of the analogues, MBI 11F3CN, is significantly less lytic than the other two variants at all three concentrations tested.

A combination of cationic peptide and antibiotic agent is tested for toxicity towards eukaryotic cells by measuring the extent of lysis of mammalian red blood cells. Briefly, red blood cells are separated from whole blood by centrifugation, washed free of plasma components, and resuspended to a 5% (v/v) suspension in isotonic saline. The peptide and antibiotic agent are pre-mixed in isotonic saline, or other acceptable solution, and an aliquot of this solution is added to the red blood cell suspension. Following incubation with constant agitation at 37°C for 1 hour, the solution is centrifuged, and the absorbance of the supernatant is measured at 540 nm, which detects released hemoglobin. Comparison to the A_{540} for a 100% lysed standard provides a relative measure of hemoglobin release from

red blood cells, indicating the lytic ability of the cationic peptide and antibiotic agent combination.

EXAMPLE 10

PHARMACOLOGY OF CATIONIC PEPTIDES IN PLASMA AND BLOOD

The *in vitro* lifetime of free peptides in plasma and in blood is determined by measuring the amount of peptide present after set incubation times. Blood is collected from sheep, treated with an anticoagulant (not heparin) and, for plasma preparation, centrifuged to remove cells. Formulated peptide is added to either the plasma fraction or to whole blood and incubated. Following incubation, peptide is identified and quantified directly by reversed phase HPLC or an antibody-based assay. The antibiotic agent is quantified by a suitable assay, selected on the basis of its structure. Chromatographic conditions are as described above. Extraction is not required as the free peptide peak does not overlie any peaks from blood or plasma.

A 1 mg/mL solution of MBI 11B7CN in isotonic saline is added to freshly prepared heat-inactivated rabbit serum, to give a final peptide concentration of 100 µg/mL and is incubated at 32°C. The peptide levels detected at various incubation times are shown in Figure 2.

A series of peptide stability studies are performed to investigate the action of protease inhibitors on peptide degradation. Peptide is added to rabbit serum or plasma, either with or without protease inhibitors, then incubated at 22°C for 3 hrs. Protease inhibitors tested include amastatin, bestatin, COMPLETE protease inhibitor cocktail, leupeptin, pepstatin A and EDTA. Amastatin and bestatin at 100 µM prevent the degradation of MBI 11B7CN in plasma over 3 hrs. For this experiments 10 mM stock solutions of amastatin and bestatin are prepared in dimethylsulfoxide. These solutions are diluted 1:100 in heat-inactivated rabbit serum and incubated at 22°C for 15 mins prior to addition of peptide. MBI 11B7CN is added to the serum at a final concentration of 100 µg/mL and incubated for 3 hrs at 22°C. After the incubation period, the serum samples are analyzed on an analytical C_8 column (Waters Nova Pak C_8 3.9 x 170 mm) with detection at 280 nm. In Figure 3, MBI 11B7CN elutes at 25 min and shows differing degrees of degradation.

Peptide is extracted from plasma using C_8 Sep Pak cartridges at peptide concentrations between 0 and 50 μg/mL. Each extraction also contains MBI 11CN at 10 μg/mL as an internal standard. Immediately after addition of the peptides to fresh rabbit plasma, the samples are mixed then diluted 1:10 with a 1% aqueous trifluoroacetic acid (TFA) solution, to give a final TFA concentration of 0.1%. Five hundred μL of this solution is immediately loaded onto a C_8 Sep Pak cartridge and eluted with 0.1% TFA in 40% acetonitrile/60% H_2O. Twenty μL of this eluant is loaded onto a 4.6 x 45 mm analytical C_{18} column and is eluted with an acetonitrile gradient of 25% to 65% over 8 column volumes. The peptides are detected at 280 nm. As shown in Figure 4, MBI 11B7CN and MBI 11CN elute at 5 and 3 min respectively. Moreover, MBI 11B7CN is detected over background at concentrations of 5 μg/mL and above.

The *in vivo* lifetime of the cationic peptide and antibiotic agent combination is determined by administration, typically by intravenous or intraperitoneal injection, of 80-100% of the maximum tolerable dose of the combination in a suitable animal model, typically a mouse. At set times post-injection, each group of animals are anesthetized, blood is drawn, and plasma obtained by centrifugation. The amount of peptide or agent in the plasma supernatant is analyzed as for the *in vitro* determination. (Figure 5).

EXAMPLE 11

TOXICITY OF CATIONIC PEPTIDES *IN VIVO*

The acute, single dose toxicity of various indolicidin analogues is tested in Swiss CD1 mice using various routes of administration. In order to determine the inherent toxicities of the peptide analogues in the absence of any formulation/delivery vehicle effects, the peptides are all administered in isotonic saline with the final pH between 6 and 7.

Intraperitoneal route. Groups of 6 mice are injected with peptide doses of between 80 and 5 mg/kg in 500 μl dose volumes. After peptide administration, the mice are observed for a period of 5 days, at which time the dose causing 50% mortality (LD_{50}), the dose causing 90-100% mortality (LD_{90-100}) and maximum tolerated dose (MTD) levels are determined. The LD_{50} values are calculated using the method of Reed and Muench (*J. of Amer. Hyg. 27*: 493-497, 1938). The results presented in Table 22 show that the LD_{50} values for MBI 11CN and analogues range from 21 to 52 mg/kg.

Table 22

Peptide	LD_{50}	LD_{90-100}	MTD
MBI 11CN	34 mg/kg	40 mg/kg	20 mg/kg
MBI 11B7CN	52 mg/kg	>80 mg/kg	30 mg/kg
MBI 11E3CN	21 mg/kg	40 mg/kg	<20 mg/kg
MBI 11F3CN	52 mg/kg	80 mg/kg	20 mg/kg

The single dose toxicity of a cationic peptide and antibiotic agent combination is examined in outbred ICR mice. Intraperitoneal injection of the combination in isotonic saline is carried out at increasing dose levels. The survival of the animals is monitored for 7 days. The number of animals surviving at each dose level is used to determine the maximum tolerated dose (MTD). In addition, the MTD can be determined after administration of the peptide and agent by different routes, at different time points, and in different formulations.

Intravenous route. Groups of 6 mice are injected with peptide doses of approximately 20, 16, 12, 8, 4 and 0 mg/kg in 100 µl volumes (4 ml/kg). After administration, the mice are observed for a period of 5 days, at which time the LD_{50}, LD_{90-100} and MTD levels are determined. The results from the IV toxicity testing of MBI 11CN and three analogues are shown in Table 23. The LD_{50}, LD_{90-100} and MTD values range from 5.8 to 15 mg/kg, 8 to 20 mg/kg and <4 to 12 mg/kg respectively.

Table 23

Peptide	LD_{50}	LD_{90-100}	MTD
MBI 11CN	5.8 mg/kg	8.0 mg/kg	<4 mg/kg
MBI 11B7CN	7.5 mg/kg	16 mg/kg	4 mg/kg
MBI 11F3CN	10 mg/kg	12 mg/kg	8 mg/kg
MBI 11F4CN	15 mg/kg	20 mg/kg	12 mg/kg

In addition, mice are multiply injected by an intravenous route with MBI 11CN. In one representative experiment, peptide administered in 10 injections of 0.84 mg/kg at 5 minute intervals is not toxic. However, two injections of peptide at 4.1 mg/kg administered with a 10 minute interval results in 60% toxicity.

Subcutaneous route. The toxicity of MBI 11CN is also determined after subcutaneous (SC) administration. For SC toxicity testing, groups of 6 mice are injected with peptide doses of 128, 96, 64, 32 and 0 mg/kg in 300 µL dose volumes (12 mL/kg). After administration, the mice are observed for a period of 5 days. None of the animals died at any

of the dose levels within the 5 day observation period. Therefore, the LD_{50}, $LD_{90\text{-}100}$ and MTD are all taken to be greater than 128 mg/kg. Mice receiving higher dose levels showed symptoms similar to those seen after IV injection suggesting that peptide entered the systemic circulation. These symptoms are reversible, disappearing in all mice by the second day of observations.

To assess the impact of dosing mice with peptide analogue, a series of histopathology investigations can be carried out. Groups of mice are administered analogue at dose levels that are either at, below the MTD, or above the MTD, a lethal dose. Multiple injections may be used to mimic possible treatment regimes. Groups of control mice are not injected or injected with buffer only.

Following injection, mice are sacrificed at specified times and their organs immediately placed in a 10% balanced formalin solution. Mice that die as a result of the toxic effects of the analogue also have their organs preserved immediately. Tissue samples are taken and prepared as stained micro-sections on slides which are then examined microscopically. Damage to tissues is assessed and this information can be used to develop improved analogues, improved methods of administration or improved dosing regimes.

Mice given a non-lethal dose are always lethargic, with raised fur and evidence of edema and hypertension, but recover to normal within two hours. Tissues from these animals indicate that there is some damage to blood vessels, particularly within the liver and lung at both the observation times, but other initial abnormalities returned to normal within the 150 minute observation time. It is likely that blood vessel damage is a consequence of continuous exposure to high circulating peptide levels.

In contrast, mice given a lethal dose have completely normal tissues and organs, except for the liver and heart of the ip and iv dosed mice, respectively. In general, this damage is identified as disruption of the cells lining the blood vessels. It appears as though the rapid death of mice is due to this damage, and that the peptide did not penetrate beyond that point. Extensive damage to the hepatic portal veins in the liver and to the coronary arterioles in the heart ias observed.

EXAMPLE 12

IN VIVO EFFICACY OF CATIONIC PEPTIDES

Cationic peptides are tested for their ability to rescue mice from lethal bacterial infections. The animal model used is an intraperitoneal (ip) inoculation of mice with 10^6-10^8 Gram-positive organisms with subsequent administration of peptide. The three pathogens investigated, methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), or *S. epidermidis*, are injected ip into mice. For untreated mice, death occurs within 12-18 hours with MSSA and *S. epidermis* and within 6-10 hours with MRSA.

Peptide is administered by two routes, intraperitoneally, at one hour post-infection, or intravenously, with single or multiple doses given at various times pre- and post-infection.

MSSA infection. In a typical protocol, groups of 10 mice are infected intraperitoneally with a $LD_{90\text{-}100}$ dose (5.2 x 10^6 CFU/mouse) of MSSA (Smith, ATCC # 19640) injected in brain-heart infusion containing 5% mucin. This strain of *S. aureus* is not resistant to any common antibiotics. At 60 minutes post-infection, formulated MBI 10CN or MBI 11CN, is injected intraperitoneally at a range of dose levels. An injection of formulation alone serves as a negative control and administration of ampicillin serves as a positive control. The survival of the mice is monitored at 1, 2, 3 and 4 hrs post-infection and twice daily thereafter for a total of 8 days.

MBI 10CN is maximally active against MSSA (70-80% survival) at doses of 15 to 38 mg/kg, although 100% survival is not achieved. Below 15 mg/kg, there is clear dose-dependent survival. At these lower dose levels, there appears to be an animal-dependent threshold, as the mice either die by day 2 or survive for the full eight day period. MBI 11CN, on the other hand, rescued 100% of the mice from MSSA infection at a dose level of 36 mg/kg, and was therefore as effective as ampicillin. There was little or no activity at any of the lower dose levels, which indicates that a minimum bloodstream peptide level must be achieved during the time that bacteria are a danger to the host.

S. epidermidis infection. Peptide analogues generally have lower MIC values against *S. epidermidis in vitro*, therefore, lower blood peptide levels might be more effective against infection.

In a typical protocol, groups of 10 mice are injected intraperitoneally with an LD_{90-100} dose (2.0×10^8 CFU/mouse) of *S. epidermidis* (ATCC # 12228) in brain-heart infusion broth containing 5% mucin. This strain of *S. epidermidis* is 90% lethal after 5 days. At 15 mins and 60 mins post-infection, various doses of formulated MBI 11CN are injected intravenously via the tail vein. An injection of formulation only serves as the negative control and injection of gentamicin serves as the positive control; both are injected at 60 minutes post-infection. The survival of the mice is monitored at 1, 2, 3, 4, 6 and 8 hrs post-infection and twice daily thereafter for a total of 8 days.

MBI 11CN prolongs the survival of the mice. Efficacy is observed at all three dose levels with treatment 15 minutes post-infection, however, there is less activity at 30 minutes post-infection and no significant effect at 60 minutes post-infection. Time of administration appears to be important in this model system, with a single injection of 6mg/kg 15 minutes post-infection giving the best survival rate.

MRSA infection. MRSA infection, while lethal in a short period of time, requires a much higher bacterial load than MSSA. In a typical protocol, groups of 10 mice are injected intraperitoneally with a LD_{90-100} dose (4.2×10^7 CFU/mouse) of MRSA (ATCC # 33591) in brain-heart infusion containing 5% mucin. The MBI 11CN treatment protocols are as follows, with the treatment times relative to the time of infection:

- 0 mg/kg — Formulation alone (negative control), injected at 0 mins
- 5 mg/kg — Three 5.5 mg/kg injections at -5, +55, and +115 mins
- 1 mg/kg (2 hr) — Five 1.1 mg/kg injections at -5, +55, +115, +175 and +235 mins
- 1 mg/kg (20 min) — Five 1.1 mg/kg injections at -10, -5, 0, +5, and +10 mins
- Vancomycin — (positive control) injected at 0 mins

Survival of mice is recorded at 1, 2, 3, 4, 6, 8, 10, 12, 20, 24 and 30. hrs post-infection and twice daily thereafter for a total of 8 days. There was no change in the number of surviving mice after 24 hrs. The 1 mg/kg (20 min) treatment protocol, with injections 5 minutes apart centered on the infection time, delayed the death of the mice to a significant extent with one survivor remaining at the end of the study.

It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made

without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

We claim:

1. An indolicidin analogue selected from the group consisting of:

MBI 11A9: Ile Leu Arg Trp Pro Trp Trp Pro Trp Trp Pro Trp Arg Arg Lys;
MBI 11A10:Trp Trp Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys;
MBI 11B19:Ile Leu Arg Trp Pro Trp Arg Arg Trp Pro Trp Arg Arg Lys;
MBI 11B20:Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Met Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Met Ala Ala;
MBI 11D19: Cys Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys;
MBI 11F5: Ile Leu Arg Arg Trp Val Trp Trp Val Trp Arg Arg Lys;
MBI 11F6: Ile Leu Arg Trp Trp Val Trp Trp Val Trp Trp Arg Arg Lys;
MBI 11G25: Leu Arg Trp Trp Trp Pro Trp Arg Arg Lys;
MBI 11G26: Leu Arg Trp Pro Trp Trp Pro Trp;
MBI 11G28: Arg Trp Trp Trp Pro Trp Arg Arg Lys;
MBI 11J01: Arg Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Arg; and
MBI 11J02 Trp Arg Trp Trp Lys Pro Lys Trp Arg Trp Pro Lys Trp;

2. An indolicidin analogue selected from the group consisting of: MBI 11G24: Leu Trp Pro Trp Trp Pro Trp Arg Arg Lys and MBI 11G27: Trp Pro Trp Trp Pro Trp Arg Arg Lys.

3. The indolicidin analogue according to either of claims 1 or 2, wherein the analogue has one or more amino acids altered to a corresponding D-amino acid.

4. The indolicidin analogue according to claim 3, wherein the N-terminal and/or C-terminal amino acid is a D-amino acid.

5. The indolicidin analogue according to any one of claims 1-4, wherein the analogue is acetylated at the N-terminal amino acid.

6. The indolicidin analogue according to any one of claims 1-5, wherein the analogue is amidated at the C-terminal amino acid.

7. The indolicidin analogue according to any one of claims 1-5, wherein the analogue is esterified at the C-terminal amino acid.

8. The indolicidin analogue according to any one of claims 1-5, wherein the analogue is modified by incorporation of homoserine/homoserine lactone at the C-terminal amino acid.

9. An isolated nucleic acid molecule whose sequence comprises one or more coding sequences of an indolicidin analogue according to either of claims 1 or 2.

10. An expression vector comprising a promoter in operable linkage with the nucleic acid molecule of claim 9.

11. A host cell transfected or transformed with the expression vector of claim 10.

12. A pharmaceutical composition comprising at least one indolicidin analogue according to any of claims 1-8 and a physiologically acceptable buffer.

13. The pharmaceutical composition according to claim 12, further comprising an antibiotic agent.

14. The pharmaceutical composition according to claim 13, wherein the antibiotic is selected from the group consisting of penicillins, cephalosporins, carbacephems, cephamycins, carbapenems, monobactams, quinolones, tetracyclines, aminoglycosides, macrolides, glycopeptides, chloramphenicols, glycylcyclines, licosamides and fluoroquinolones.

15. A pharmaceutical composition comprising a physiologically acceptable buffer and a combination of a cationic peptide and an antibiotic, wherein the combination is selected from the group consisting of:

Ile Leu Lys Lys Phe Pro Phe Phe Pro Phe Arg Arg Lys and ciprofloxacin.

Ile Leu Lys Lys Phe Pro Phe Phe Pro Phe Arg Arg Lys and vancomycin.

Ile Leu Arg Arg Trp Pro Trp Trp Pro Trp Arg Arg Arg and piperacillin.

Ile Leu Arg Trp Pro Trp Trp Pro Trp Arg Arg Lys Ile Met Ile Leu Lys Lys Ala Gly Ser and gentamicin.

Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp and vancomycin.

Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp and tobramycin.

Trp Arg Ile Trp Lys Pro Lys Trp Arg Leu Pro Lys Trp and piperacillin.

ile Leu Lys Lys Trp Pro Trp Trp Pro Trp Arg Arg lys and piperacillin.

Ile Leu Lys Lys Trp Val Trp Trp Pro Trp Arg Arg Lys and tobramycin and

Ile Leu Arg Trp Val Trp Trp Val Trp Arg Arg Lys and piperacillin.

16. A device coated with a composition comprising a cationic peptide and an antibiotic agent.

17. The device of claim 16, wherein the device is a medical device.

18. A method of overcoming tolerance of a bacterium to an antibacterial agent, comprising: contacting the bacterium with a composition comprising the antibacterial agent and a cationic peptide, therefrom overcoming tolerance.

19. The method of claim 18, wherein the cationic peptide is selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, BPTI, Brevinins, CAP 18 and related peptides, Cecropins, Ceratotoxins, Charybdtoxins, Coleoptericins, Crabolins, alpha, beta, and insect defensins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Lactoferricins, Lantibiotics, Leukocins, Magainins and related peptides from amphibians, Mastoparans, Melittins, Phormicins, Polyphemusins, Protegrins, Royalisins, Sarcotoxins, Seminal plasmins, Sepacins, Tachyplesins, Thionins, Toxins, Cecropin-Melittin chimeras and analogues thereof.

20. The method of claim 18, wherein the cationic peptide is an indolicidin analogue.

21. A method of overcoming inherent resistance of a microorganism to an antibiotic agent, comprising: contacting the microorganism with a composition comprising the antibiotic agent and a cationic peptide selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, Brevinins, CAP 18 and related peptides, Cecropins, Ceratotoxins, Charybdtoxins, Coleoptericins, Crabolins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Lactoferricins, Lantibiotics, Leukocins, Magainins and related peptides from amphibians, Mastoparans, Melittins, Phormicins, Polyphemusins, Protegrins, Royalisins, Sarcotoxins, Seminal plasmins, Sepacins, Tachyplesins, Thionins, Toxins, Cecropin-Melittin chimeras and analogues thereof, therefrom overcoming inherent resistance.

22. The method of claim 21, wherein the cationic peptide is an indolicidin analogue.

23. A method of overcoming acquired resistance of a microorganism to an antibiotic agent, comprising: contacting the microorganism with a composition comprising the antibiotic agent and a cationic peptide selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, Brevinins, CAP 18 and related peptides, Cecropins, Ceratotoxins, Charybdtoxins, Coleoptericins, Crabolins, alpha, beta, and insect Defensins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Lactoferricins, Lantibiotics, Leukocins, Magainins and related peptides from amphibians, Mastoparans, Melittins, Phormicins, Protegrins, Royalisins, Sarcotoxins, Seminal plasmins, Sepacins, Thionins, Toxins, Cecropin-Melittin chimeras and analogues thereof, therefrom overcoming acquired resistance .

24. The method of claim 23, wherein the cationic peptide is an indolicidin analogue.

25. A method of overcoming tolerance of a bacterium to an antibacterial agent, overcoming inherent resistance of a microorganism an antibacterial agent, overcoming acquired resistance of a microorganism an antibacterial agent or enhancing the activity of an antibiotic agent against a susceptible microorganism, comprising administering a pharmaceutical composition of lysozyme or nisin and an antibacterial agent, therefrom overcoming tolerance, inherent resistance, acquired resistance, or enhancing activity.

26. A method of enhancing activity of an antibiotic agent against a susceptible microorganism, comprising administering a pharmaceutical composition comprising the antibiotic agent and a cationic peptide selected from the group consisting of Abaecins, Andropins, Apidaecins, AS, Bactenecins, Bac, Bactericidins, Bacteriocins, Bombinins, Bombolitins, Brevinins, CAP 18 and related peptides, Ceratotoxins, Charybdtoxins, Coleoptericins, alpha, beta, and insect Defensins, Dermaseptins, Diptericins, Drosocins, Esculentins, Gramicidins, Histatins, Indolicidins, Leukocins, Mastoparans, Phormicins, Polyphemusins, Protegrins, Royalisins, Seminal plasmins, Sepacins, Thionins, Toxins and analogues thereof, therefrom enhancing activity of the antibiotic agent against the susceptible microorganism.

27. The method of claim 26, wherein the cationic peptide is an indolicidin analogue.

28. The method of claim 19, wherein the cationic peptide and antibacterial agents are selected from the group consisting of MBI 11A1CN and Chloramphenicol; MBI 11B4CN and Erythromycin; MBI 21A10 and Ampicillin; MBI 21A10 and Piperacillin; MBI 26 and Vancomycin; MBI 29 and Gentamicin and MBI 29A3 and Penicillin.

29. The method of claim 21, wherein the cationic peptide and antibiotic agents are selected from the group consisting of MBI 11B16CN and Amikacin; MBI 11D18CN and Gentamicin; MBI 11D18CN and Gentamicin; MBI 21A1 and Mupirocin; MBI 21A1 and Tobramycin; MBI 26 and Amikacin; MBI 26 and Gentamicin; MBI 29A3 and Amikacin; MBI 29A3 and Tobramycin and MBI 29F1 and Amikacin.

30. The method of claim 23, wherein the cationic peptide and antibiotic agent are selected from the group consisting of MBI 11A1CN and Vancomycin; MBI 11B16CN and Gentamicin; MBI 11D18CN and Gentamicin; MBI 11F3CN and Tobramycin; MBI 11F4CN and Piperacillin; MBI 21A1 and Tobramycin; MBI 26 and Ceftriaxone; MBI 26 and Vancomycin; MBI 29A2 and Ciprofloxacin and MBI 29A3 and Ciprofloxacin.

31. The method of claim 26, wherein the cationic peptide and antibiotic agent are selected from the group consisting of MBI 11B16CN and Amikacin; MBI 11CN and Piperacillin; MBI 11G13CN and Tobramycin; MBI 11G7CN and Piperacillin; MBI 11J02CN

and Ceftriaxone; MBI 21A2 and Gentamicin; MBI 28 and Mupirocin; MBI 29 and Vancomycin; MBI 29A2 and Ciprofloxacin and REWH 53A5CN and Tobramycin.

32. The method of any of claims 18-31, wherein the infection is due to a microorganism.

33. The method of claim 32, wherein the microorganism is selected from the group consisting of bacterium, fungus, parasite and virus.

34. The method of claim 33, wherein the fungus is a yeast and/or mold.

35. The method of claim 33, wherein the parasite is selected from the group consisting of protozoan, nematode, cestode and trematode.

36. The method of claim 35, wherein the parasite is selected from the group consisting of *Babesia spp.; Balantidium coli; Blastocystis hominis; Cryptosporidium parvum; Encephalitozoon spp.; Entamoeba spp.; Giardia lamblia; Leishmania spp.; Plasmodium spp.; Toxoplasma gondii; Trichomonas spp.; Trypanosoma spp; Ascaris lumbricoides; Clonorchis sinensis; Echinococcus spp.; Fasciola hepatica; Fasciolopsis buski; Heterophyes heterophyes; Hymenolepis spp.; Schistosoma spp.; Taenia spp.* and *Trichinella spiralis.*

37. The method of claim 33, wherein the bacterium is a Gram-negative bacterium.

38. The method of claim 37, wherein the Gram-negative bacterium is selected from the group consisting of *Acinetobacter spp.; Enterobacter spp.; E. coli; H. influenzae;, K. pneumoniae; P. aeruginosa; S. marcescens; S. maltophilia; Bordetella pertussis; Brucella spp.; Campylobacter spp.; Haemophilus ducreyi; Helicobacter pylori; Legionella spp.; Moraxella catarrhalis; Neisseria spp.; Salmonella spp.; Shigella spp.* and *Yersinia spp.*

39. The method of claim 33, wherein the bacterium is a Gram-positive bacterium.

40. The method of claim 39, wherein the Gram-positive bacterium is selected from the group consisting of *E. faecalis; S. aureus; E. faecium; S. pyogenes; S. pneumoniae;* coagulase-negative staphylococci; *Bacillus spp.; Corynebacterium spp.;* Diphtheroids; *Listeria spp.* and Viridans Streptococci.

41. The method of claim 33, wherein the bacterium is an anaerobe selected from the group consisting *Clostridium spp., Bacteroides spp.* and *Peptostreptococcus spp.*

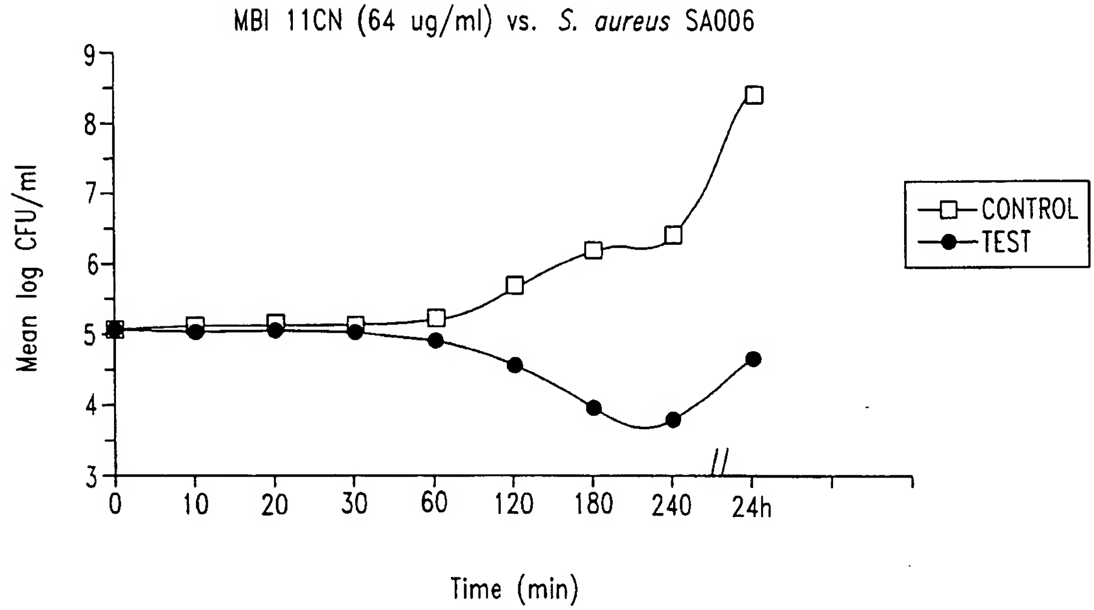
42. The method of claim 33, wherein the bacterium is selected from the group consisting of *Borrelia spp.; Chlamydia spp.; Mycobacterium spp.; Mycoplasma spp.; Propionibacterium acne; Rickettsia spp.; Treponema spp.* and *Ureaplasma spp.*

44. The method of claim 33, wherein the virus is an RNA virus selected from the group consisting of Alphavirus; Arenavirus; Bunyavirus; Coronavirus; Enterovirus; Filovirus; Flavivirus; Hantavirus; HTLV-BLV; Influenzavirus; Lentivirus; Lyssavirus; Paramyxovirus; Reovirus; Rhinovirus and Rotavirus.

45. The method of claim 33, wherein the virus is a DNA virus selected from the group consisting of Adenovirus; Cytomegalovirus; Hepadnavirus; Molluscipoxvirus; Orthopoxvirus; Papillomavirus; Parvovirus; Polyomavirus; Simplexvirus and Varicellovirus.

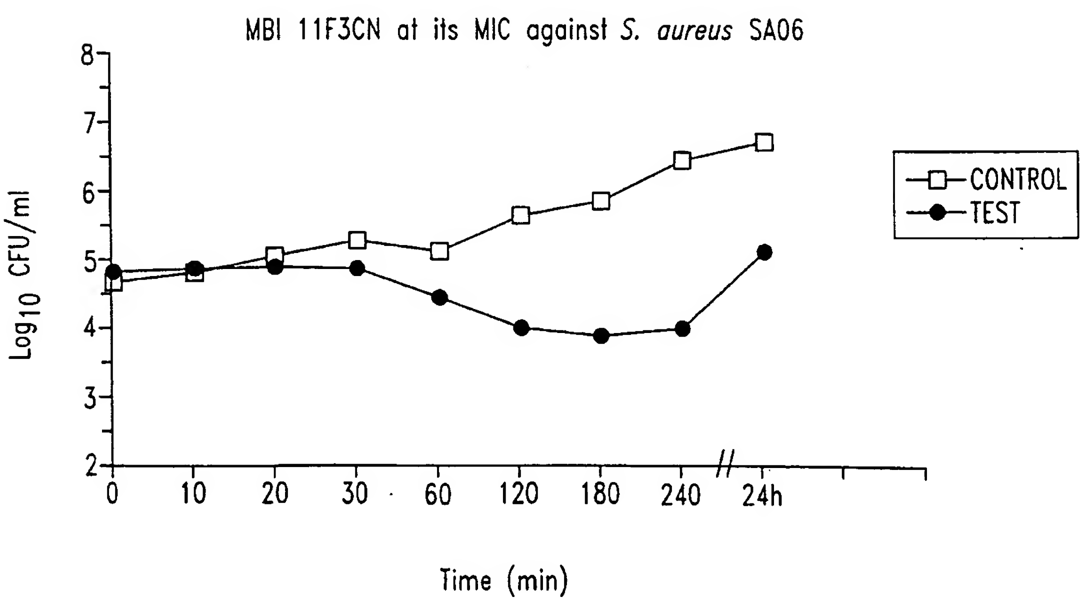
46. The method of any of claims 18-45, wherein the pharmaceutical composition is administered by intravenous injection, intraperitoneal injection or implantation, intramuscular injection or implantation, intrathecal injection, subcutaneous injection or implantation, intradermal injection, lavage, bladder wash-out, suppositories,

pessaries, oral ingestion, topical application, enteric application, inhalation, aerosolization or nasal spray or drops.



MBI 11CN (64 ug/ml) vs. S. aureus SA006
Mean log CFU/ml
9
8
7
6
5
4
3
0
10
20
30
60
120
180
240
24h
Time (min)
CONTROL
TEST

Fig. 1A



MBI 11F3CN at its MIC against S. aureus SA06
Log10 CFU/ml
8
7
6
5
4
3
2
0
10
20
30
60
120
180
240
24h
Time (min)
CONTROL
TEST

Fig. 1B

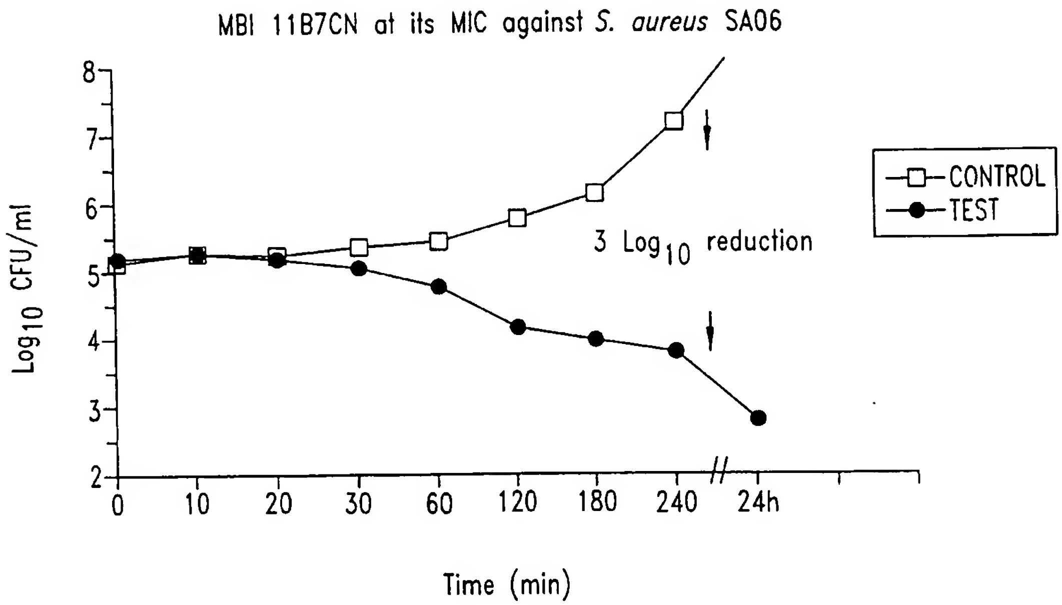

MBI 11B7CN at its MIC against S. aureus SA06
8
7
6
5
4
3
2
Log_{10} CFU/ml
0
10
20
30
60
120
180
240
24h
Time (min)
3 Log_{10} reduction
CONTROL
TEST

Fig. 1C

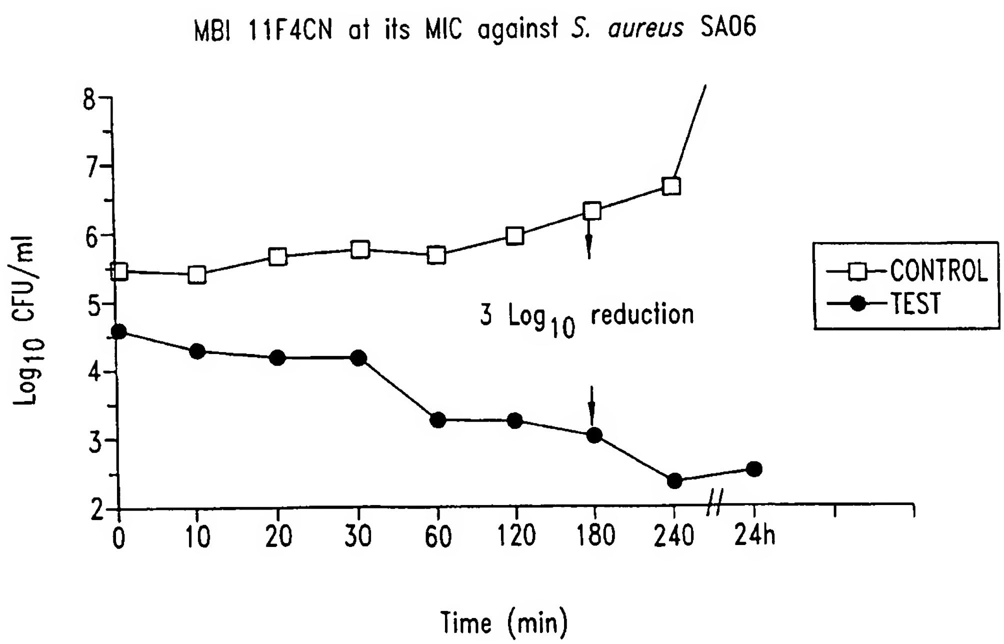

MBI 11F4CN at its MIC against S. aureus SA06
8
7
6
5
4
3
2
Log_{10} CFU/ml
0
10
20
30
60
120
180
240
24h
Time (min)
3 Log_{10} reduction
CONTROL
TEST

Fig. 1D

MBI 26 & Vancomycin in Combination Against *E. faecium* EFM017

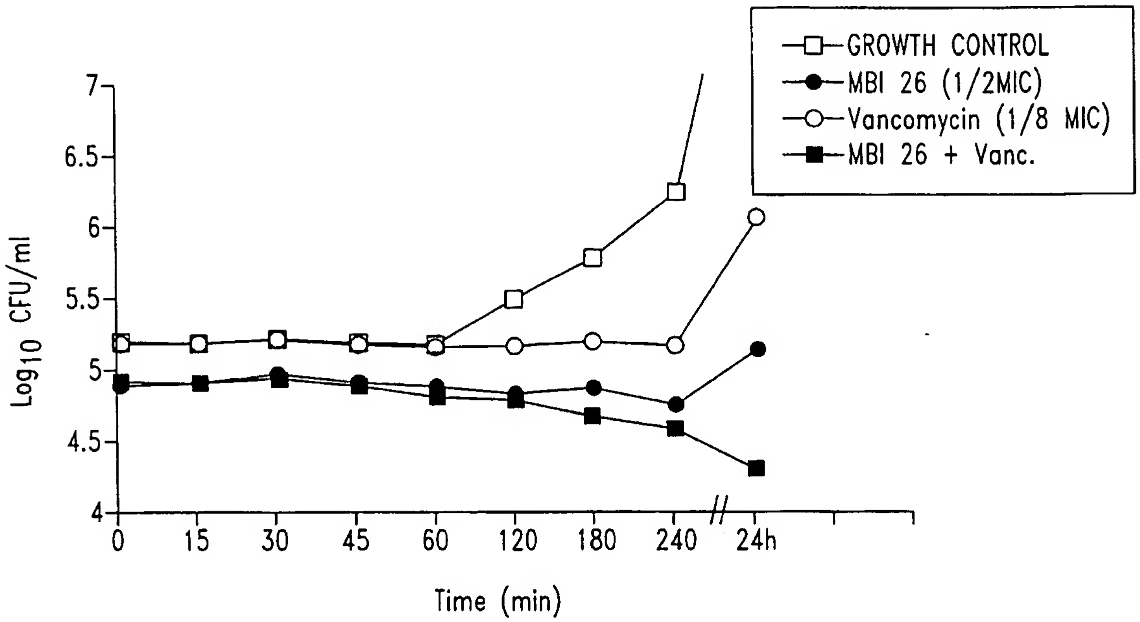


Fig. 1E

Stability of MBI-11B7CN-Cl in Heat-inactivated Rabbit Serum

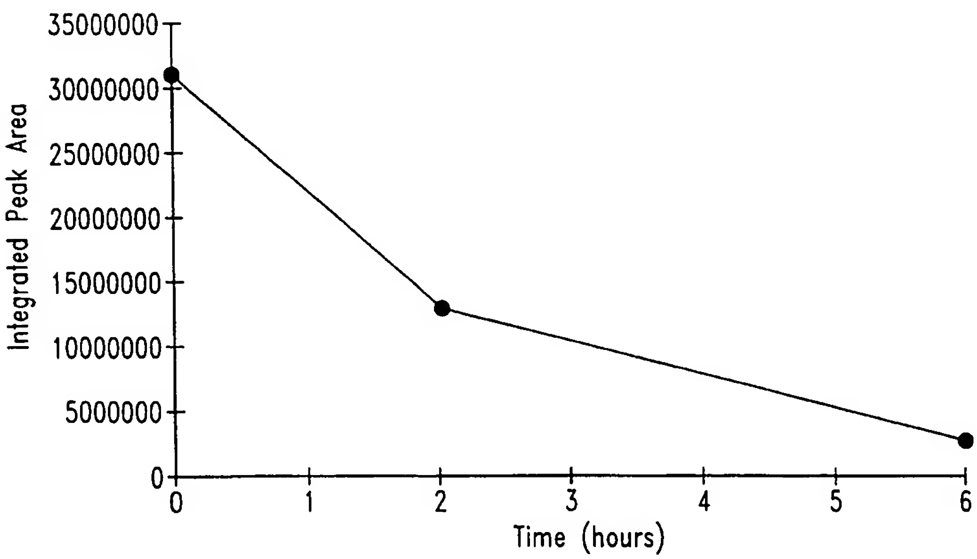


Fig. 2

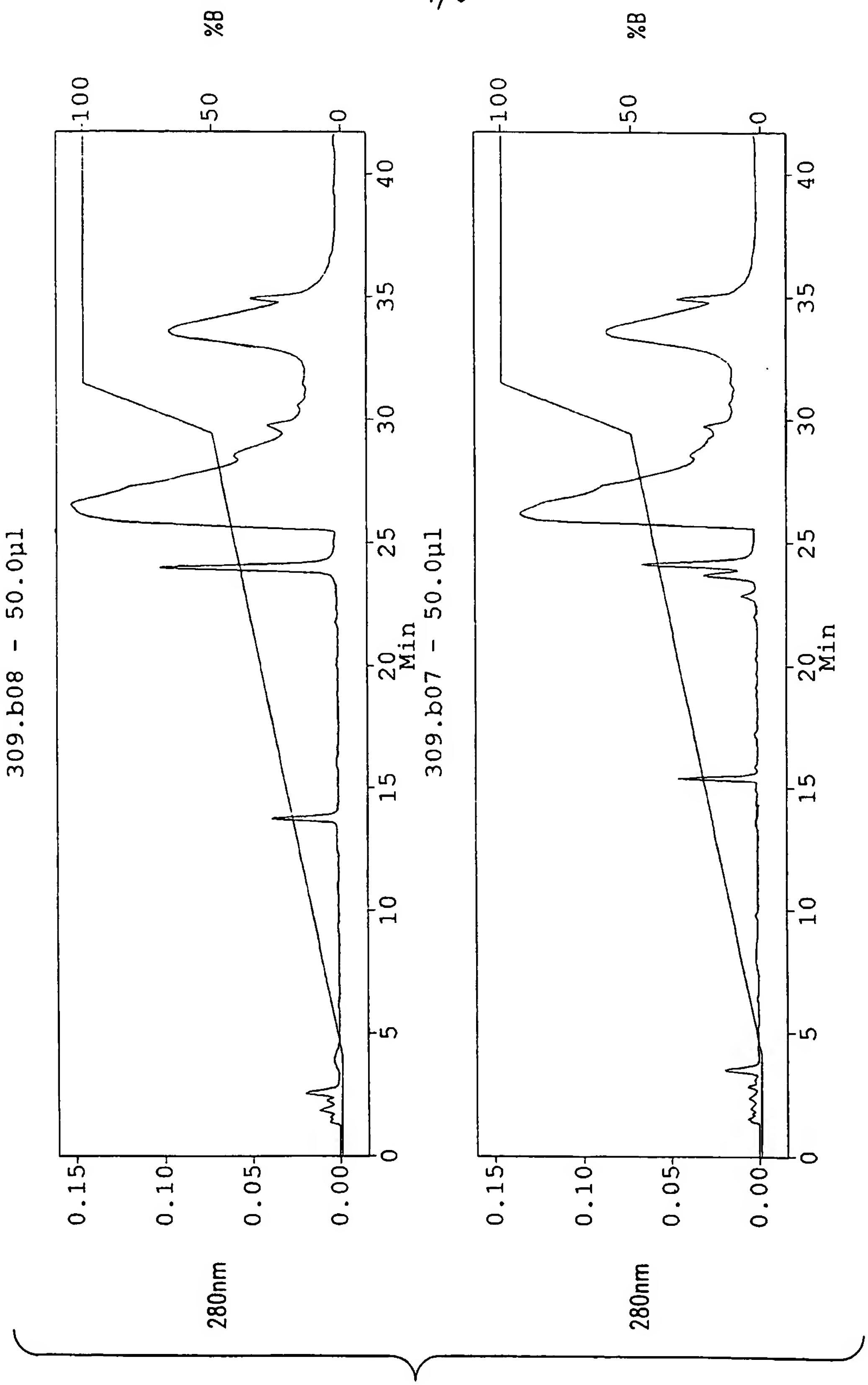

309.b08 - 50.0µl
280nm
%B
0.15
0.10
0.05
0.00
100
50
0
0
5
10
15
20
25
30
35
40
Min
309.b07 - 50.0µl
280nm
%B
0.15
0.10
0.05
0.00
100
50
0
0
5
10
15
20
25
30
35
40
Min

Fig. 3


0.0100
0.0075
0.0050
0.0025
0.0000
280nm
312.b73 - 20.0µl
312.b72 - 20.0µl
312.b71 - 20.0µl
312.b70 - 20.0µl
312.b69 - 20.0µl
0
5
10
Min

Fig. 4

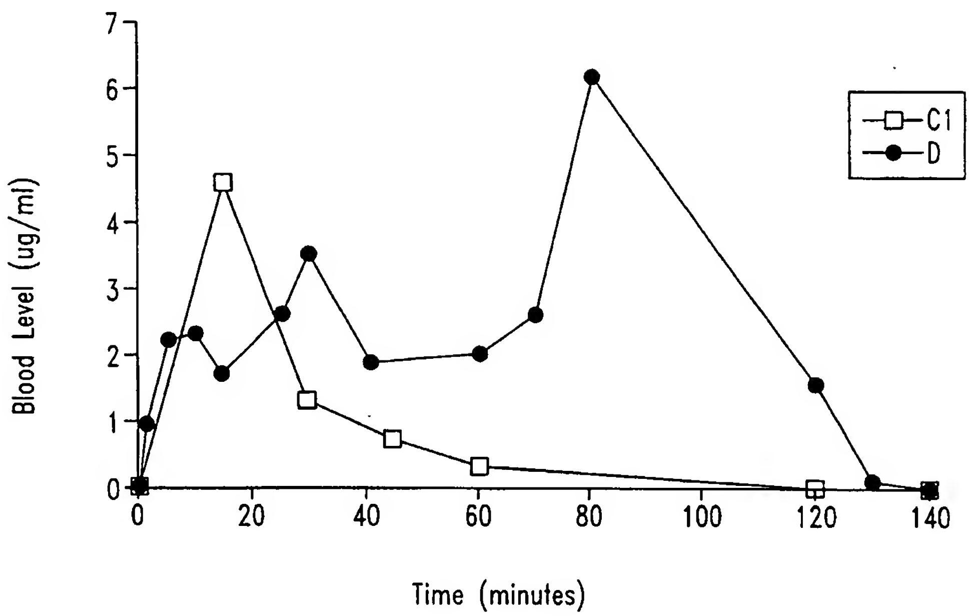

Blood Level (ug/ml)
Time (minutes)
C1
D
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120
140

Fig. 5